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Patent Application
Washington, D. C. 20231

Honorable Sir:

Transmitted herewith for filing under 37 CFR 1.53(b) is the:
☒ patent application, ☐ continuation patent
application, ☐ divisional patent application
☐ continuation-in-part (CIP) patent application of:

**HUMAN RECEPTOR PROTEINS; RELATED
REAGENTS AND METHODS**

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Application, Washington, D. C. 20231.

By Lois E. Miller March 8, 1999
Lois E. Miller March 8, 1999

Inventors: **Christi L. Parham, Kevin W. Moore, Nicholas J. Murgolo and J. Fernando Bazan**

Sir:

1. ☒ This application claims priority from each of the following Application Nos./filing dates:
U.S. Provisional Application number 60/077,329, filed March 9, 1998.
2. ☐ Please amend the specification by replacing the first paragraph with the following:
3. Enclosed are:

- ☒ RETURN RECEIPT POSTCARD
- ☒ PATENT APPLICATION TRANSMITTAL EL 263 586 741 US: 1 Page (in duplicate)
- ☒ DECLARATION BY INVENTORS/POWER OF ATTORNEY: 3 Pages: ☐ Signed ☒ Unsigned
- ☒ PATENT APPLICATION TITLE PAGE: 1 Page (unnumbered)
- ☒ SPECIFICATION: 76 Pages: 1 through 76
- ☒ CLAIMS (20 claims): 9 Pages: 77 through 85
- ☒ SEQUENCE LISTING: 11 Page(s): 86 through 96
- ☒ ABSTRACT: 1 Page: page 97
- ☒ Sequence Transmittal (2 pages), diskette and paper print-out (11 pages)

**In view of the Unsigned Declaration as filed with this application,
Applicant requests deferral of the filing fee until submission of
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Respectfully submitted,
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Edwin P. Ching March 8, 1999
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PATENT APPLICATION

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HUMAN RECEPTOR PROTEINS; RELATED REAGENTS AND METHODS

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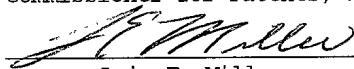
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HUMAN RECEPTOR PROTEINS; RELATED REAGENTS AND METHODS

This filing is a conversion of U.S. Provisional Patent Application 60/077,329, filed March 9, 1998, which is
5 incorporated herein by reference, to a U.S. Utility Patent Application.

FIELD OF THE INVENTION

The present invention relates to compositions and
10 methods for affecting mammalian physiology, including morphogenesis or immune system function. In particular, it provides nucleic acids, proteins, and antibodies which regulate development and/or the immune system. Diagnostic and therapeutic uses of these materials are also disclosed.

15

BACKGROUND OF THE INVENTION

Recombinant DNA technology refers generally to techniques of integrating genetic information from a donor source into vectors for subsequent processing, such as
20 through introduction into a host, whereby the transferred genetic information is copied and/or expressed in the new environment. Commonly, the genetic information exists in the form of complementary DNA (cDNA) derived from messenger RNA (mRNA) coding for a desired protein product. The
25 carrier is frequently a plasmid having the capacity to incorporate cDNA for later replication in a host and, in some cases, actually to control expression of the cDNA and thereby direct synthesis of the encoded product in the host. See, e.g., Sambrook, et al. (1989) Molecular
30 Cloning: A Laboratory Manual, (2d ed.), vols. 1-3, CSH Press, NY.

For some time, it has been known that the mammalian immune response is based on a series of complex cellular interactions, called the "immune network". Recent research
35 has provided new insights into the inner workings of this network. While it remains clear that much of the immune response does, in fact, revolve around the network-like

interactions of lymphocytes, macrophages, granulocytes, and other cells, immunologists now generally hold the opinion that soluble proteins, known as lymphokines, cytokines, or monokines, play critical roles in controlling these cellular interactions. The interferons are generally considered to be members of the cytokine family. Thus, there is considerable interest in the isolation, characterization, and mechanisms of action of cell modulatory factors, an understanding of which will lead to significant advancements in the diagnosis and therapy of numerous medical abnormalities, e.g., immune system disorders.

Lymphokines apparently mediate cellular activities in a variety of ways. See, e.g., Paul (ed. 1996) Fundamental Immunology 3d ed., Raven Press, New York; and Thomson (ed. 1994) The Cytokine Handbook 2d ed., Academic Press, San Diego. They have been shown to support the proliferation, growth, and/or differentiation of pluripotential hematopoietic stem cells into vast numbers of progenitors comprising diverse cellular lineages which make up a complex immune system. Proper and balanced interactions between the cellular components are necessary for a healthy immune response. The different cellular lineages often respond in a different manner when lymphokines are administered in conjunction with other agents.

Cell lineages especially important to the immune response include two classes of lymphocytes: B-cells, which can produce and secrete immunoglobulins (proteins with the capability of recognizing and binding to foreign matter to effect its removal), and T-cells of various subsets that secrete lymphokines and induce or suppress the B-cells and various other cells (including other T-cells) making up the immune network. These lymphocytes interact with many other cell types.

One means to modulate the effect of a cytokine upon binding to its receptor, and therefore potentially useful in treating inappropriate immune responses, e.g., autoimmune,

inflammation, sepsis, and cancer situations, is to inhibit the receptor signal transduction. Unfortunately, finding reagents capable of serving as an antagonist or agonist has been severely hampered by the failure to fully identify all of the components within the signaling systems. In order to characterize the structural properties of a cytokine receptor in greater detail and to understand the mechanism of action at the molecular level, purified receptor will be very useful. The receptors provided herein, by comparison to other receptors or by combining structural components, will provide further understanding of signal transduction induced by ligand binding.

The isolated receptor gene should provide means to generate an economical source of the receptor, allow expression of more receptors on a cell leading to increased assay sensitivity, promote characterization of various receptor subtypes and variants, and allow correlation of activity with receptor structures. Moreover, fragments of the receptor may be useful as agonists or antagonists of ligand binding. See, e.g., Harada, et al. (1992) J. Biol. Chem. 267:22752-22758. Often, there are at least two critical subunits in the functional receptor. See, e.g., Gonda and D'Andrea (1997) Blood 89:355-369; Presky, et al. (1996) Proc. Nat'l Acad. Sci. USA 93:14002-14007; Drachman and Kaushansky (1995) Curr. Opin. Hematol. 2:22-28; Theze (1994) Eur. Cytokine Netw. 5:353-368; and Lemmon and Schlessinger (1994) Trends Biochem. Sci. 19:459-463.

From the foregoing, it is evident that the discovery and development of new soluble proteins and their receptors, including ones similar to lymphokines, should contribute to new therapies for a wide range of degenerative or abnormal conditions which directly or indirectly involve development, differentiation, or function, e.g., of the immune system and/or hematopoietic cells. In particular, the discovery and understanding of novel receptors for lymphokine-like molecules which enhance or potentiate the beneficial activities of other

lymphokines would be highly advantageous. The present invention provides new receptors for ligands exhibiting similarity to cytokine like compositions and related compounds, and methods for their use.

continued on next page

SUMMARY OF THE INVENTION

The present invention is directed to novel receptors related to cytokine receptors, e.g., primate or rodent, cytokine receptor like molecular structures, designated
5 DNAX Interferon-like Receptor Subunits (DIRS), and their biological activities. In particular, it provides description of two different subunits, designated DIRS1 and DIRS2. It includes nucleic acids coding for the polypeptides themselves and methods for their production
10 and use. The nucleic acids of the invention are characterized, in part, by their homology to cloned complementary DNA (cDNA) sequences enclosed herein.

The present invention provides, in polypeptide embodiments: a substantially pure or recombinant DIRS1
15 polypeptide comprising at least three distinct nonoverlapping segments of at least four amino acids identical to segments of SEQ ID NO: 2; a substantially pure or recombinant DIRS1 polypeptide comprising at least two distinct nonoverlapping segments of at least five amino
20 acids identical to segments of SEQ ID NO: 2; a natural sequence DIRS1 comprising mature SEQ ID NO: 2; a fusion polypeptide comprising DIRS1 sequence; a substantially pure or recombinant DIRS2 polypeptide comprising at least three distinct nonoverlapping segments of at least ten amino
25 acids identical to segments of SEQ ID NO: 4; a substantially pure or recombinant DIRS2 polypeptide comprising at least two distinct nonoverlapping segments of at least eleven amino acids identical to segments of SEQ ID NO: 4; a natural sequence DIRS2 comprising SEQ ID NO: 4; or
30 a fusion polypeptide comprising DIRS2 sequence. Preferred embodiments include, e.g., the substantially pure or isolated antigenic: DIRS1 polypeptide, wherein the distinct nonoverlapping segments of identity: include one of at least eight amino acids; include one of at least four amino
35 acids and a second of at least five amino acids; include at least three segments of at least four, five, and six amino acids, or include one of at least twelve amino acids; or

DIRS2 polypeptide, wherein the distinct nonoverlapping segments of identity: include one of at least thirteen amino acids; include one of at least eleven amino acids and a second of at least thirteen amino acids; include at least
5 three segments of at least ten, eleven, and twelve amino acids; or include one of at least twenty-five amino acids. Other embodiments include compositions where: the DIRS1 polypeptide: comprises a mature sequence of Table 1; is an unglycosylated form of DIRS1; is from a primate, such as a
10 human; comprises at least seventeen amino acids of SEQ ID NO: 2; exhibits at least four nonoverlapping segments of at least seven amino acids of SEQ ID NO: 2; is a natural allelic variant of DIRS1; has a length at least about 30 amino acids; exhibits at least two non-overlapping epitopes
15 which are specific for a primate DIRS1; is glycosylated; has a molecular weight of at least 30 kD with natural glycosylation; is a synthetic polypeptide; is attached to a solid substrate; is conjugated to another chemical moiety; is a 5-fold or less substitution from natural sequence; or
20 is a deletion or insertion variant from a natural sequence; or the DIRS2 polypeptide: comprises a mature sequence of Table 2; is an unglycosylated form of DIRS2; or is from a primate, such as a human; comprises at thirty-five amino acids of SEQ ID NO: 4; exhibits at least four
25 nonoverlapping segments of at least twelve amino acids of SEQ ID NO: 4; is a natural allelic variant of DIRS2; has a length at least about 30 amino acids; exhibits at least two non-overlapping epitopes which are specific for a primate DIRS2; is glycosylated; has a molecular weight of at least
30 30 kD with natural glycosylation; is a synthetic polypeptide; is attached to a solid substrate; is conjugated to another chemical moiety; is a 5-fold or less substitution from natural sequence; or is a deletion or insertion variant from a natural sequence. Various
35 combination compositions include those comprising: a substantially pure DIRS1 and another Interferon Receptor family member; a substantially pure DIRS2 and another

Interferon Receptor family member; a sterile DIRS1 polypeptide; a sterile DIRS2 polypeptide; the DIRS1 polypeptide and a carrier, wherein the carrier is: an aqueous compound, including water, saline, and/or buffer; and/or formulated for oral, rectal, nasal, topical, or parenteral administration; or the DIRS2 polypeptide and a carrier, wherein the carrier is: an aqueous compound, including water, saline, and/or buffer; and/or formulated for oral, rectal, nasal, topical, or parenteral administration.

Fusion polypeptide embodiments include those comprising: mature protein sequence of Table 1; mature protein sequence of Table 2; a detection or purification tag, including a FLAG, His6, or Ig sequence; or sequence of another interferon receptor protein. Kit embodiments are provided, e.g., a kit comprising such a polypeptide, and: a compartment comprising the protein or polypeptide; or instructions for use or disposal of reagents in the kit.

The invention also provides a binding compound comprising an antigen binding site from an antibody, which specifically binds to a: natural DIRS1 polypeptide, wherein: the binding compound is in a container; the DIRS1 polypeptide is from a human; the binding compound is an Fv, Fab, or Fab2 fragment; the binding compound is conjugated to another chemical moiety; or the antibody: is raised against a peptide sequence of a mature polypeptide of Table 1; is raised against a mature DIRS1; is raised to a purified human DIRS1; is immunoselected; is a polyclonal antibody; binds to a denatured DIRS1; exhibits a Kd to antigen of at least 30 μ M; is attached to a solid substrate, including a bead or plastic membrane; is in a sterile composition; or is detectably labeled, including a radioactive or fluorescent label; or a natural DIRS2 polypeptide, wherein: the binding compound is in a container; the DIRS2 protein is from a human; the binding compound is an Fv, Fab, or Fab2 fragment; the binding compound is conjugated to another chemical moiety; or the

replication; is from a natural source; comprises a detectable label; comprises synthetic nucleotide sequence; is less than 6 kb, preferably less than 3 kb; is from a primate; comprises a natural full length coding sequence; is a hybridization probe for a gene encoding the DIRS1; or is a PCR primer, PCR product, or mutagenesis primer; or the described DIRS2 polypeptide, wherein the: DIRS2 is from a human; or the nucleic acid: encodes an antigenic peptide sequence of Table 2; encodes a plurality of antigenic peptide sequences of Table 2; exhibits identity over at least 30 nucleotides to a natural cDNA encoding the segment; is an expression vector; further comprises an origin of replication; is from a natural source; comprises a detectable label; comprises synthetic nucleotide sequence; is less than 6 kb, preferably less than 3 kb; is from a primate; comprises a natural full length coding sequence; is a hybridization probe for a gene encoding the DIRS2; or is a PCR primer, PCR product, or mutagenesis primer.

The invention further provides a cell or tissue comprising the described recombinant nucleic acid. Certain embodiments include wherein the cell is: a prokaryotic cell; a eukaryotic cell; a bacterial cell; a yeast cell; an insect cell; a mammalian cell; a mouse cell; a primate cell; or a human cell. Kits are also provided, e.g., the described nucleic acid and: a compartment comprising the nucleic acid; a compartment further comprising a primate DIRS1 polypeptide; a compartment further comprising a primate DIRS2 polypeptide; or instructions for use or disposal of reagents in the kit.

In other embodiments, the invention provides a nucleic acid which: hybridizes under wash conditions of 30 minutes at 30° C and less than 2M salt to the coding portion of SEQ ID NO: 1; hybridizes under wash conditions of 30 minutes at 30° C and less than 2M salt to the coding portion of SEQ ID NO: 3; exhibits identity over a stretch of at least about 30 nucleotides to a primate DIRS1 sequence; or exhibits

identity over a stretch of at least about 30 nucleotides to a primate DIRS2 sequence. Preferred embodiments include those nucleic acids wherein: the wash conditions are at 45° C and/or 500 mM salt; or the stretch is at least 55

- 5 nucleotides. Other embodiments include those nucleic acids wherein: the wash conditions are at 55° C and/or 150 mM salt; or the stretch is at least 75 nucleotides.

- 10 The invention further provides a method of modulating physiology or development of a cell or tissue culture cells comprising contacting the cell with an agonist or antagonist of a mammalian DIRS1 or DIRS2. The method may involve where the cell is transformed with a nucleic acid encoding a DIRS1 or DIRS2 and another cytokine receptor subunit.

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DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

OUTLINE

- I. General
- 5 II. Activities
- III. Nucleic acids
- A. encoding fragments, sequence, probes
- B. mutations, chimeras, fusions
- C. making nucleic acids
- 10 D. vectors, cells comprising
- IV. Proteins, Peptides
- A. fragments, sequence, immunogens, antigens
- B. muteins
- C. agonists/antagonists, functional equivalents
- 15 D. making proteins
- V. Making nucleic acids, proteins
- A. synthetic
- B. recombinant
- C. natural sources
- 20 VI. Antibodies
- A. polyclonals
- B. monoclonal
- C. fragments; Kd
- D. anti-idiotypic antibodies
- 25 E. hybridoma cell lines
- VII. Kits and Methods to quantify DIRS
- A. ELISA
- B. assay mRNA encoding
- C. qualitative/quantitative
- 30 D. kits
- VIII. Therapeutic compositions, methods
- A. combination compositions
- B. unit dose
- C. administration
- 35 IX. Screening
- X. Ligands

I. General

The present invention provides the amino acid

40 sequences and DNA sequences of mammalian, herein primate, interferon receptor-like subunit molecules, these ones designated DNAX Interferon Receptor family Subunit 1 (DIRS1) and DNAX Interferon Receptor family Subunit 2, having particular defined properties, both structural and

45 biological. Various cDNAs encoding these molecules were obtained from primate, e.g., human, cDNA sequence libraries. Other primate or other mammalian counterparts

would also be desired. Descriptions, methods, and manipulations directed to DIRS1 may be applied, as appropriate, to DIRS2.

- Some of the standard methods applicable are described or referenced, e.g., in Maniatis, et al. (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor Press; Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual, (2d ed.), vols. 1-3, CSH Press, NY; Ausubel, et al., Biology, Greene Publishing Associates, Brooklyn, NY; or Ausubel, et al. (1987 and periodic supplements) Current Protocols in Molecular Biology, Greene/Wiley, New York; each of which is incorporated herein by reference.

- A partial nucleotide (SEQ ID NO: 1) and corresponding amino acid sequence (SEQ ID NO: 2) of a human DIRS1 coding segment is shown in Table 1. Partial human DIRS2 sequence is provided (SEQ ID NO: 3 and 4).

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Table 1: Nucleotide and amino acid sequences of DNAX IFN Receptor Subunit like embodiments (DIRS1), originally designated HKAEF92.

Primate, e.g., human embodiment (see SEQ ID NO: 1 and 2).

Nucleotides 567, 573, 1336, 1342, and 1369 are designated C, but may be A, C, G, or T; nucleotides 643, 1287, and 1290 are designated C, but may be C or G; nucleotides 772, 806, and 1261 are designated G, but may be A or G; nucleotides 1236, 1260, 1282, and 1289 are designated U, but may be G or T; residues 1247, 1257, 1293, and 1302 are designated C, but may be C or T; and nucleotides 1266 and 1298 are designated T, but may be A or T. Additional sequencing indicates that nucleotide 567 is A; 574 is G; 640 is G; 742 is G; and 806 is G. Predicted signal cleavage is about between thr29 and asp30.

15	TCGACCCACG CGTCCGCGCT GCGACTCAGA CCTCAGCTCC AACATATGCA TTCTGAAGAA	60
	AGATGGCTGA GATGGACAGA ATGCTTTATT TTGGAAAGAA ACAATGTTCT AGGTCAAAC	120
	GAGTCTACCA A ATG CAG ACT TTC ACA ATG GTT CTA GAA GAA ATC TGG ACA	170
	Met Gln Thr Phe Thr Met Val Leu Glu Glu Ile Trp Thr	
20	1 5 10	
	AGT CTT TTC ATG TGG TTT TTC TAC GCA TTG ATT CCA TGT TTG CTC ACA	218
	Ser Leu Phe Met Trp Phe Phe Tyr Ala Leu Ile Pro Cys Leu Leu Thr	
	15 20 25	
25	GAT GAA GTG GCC ATT CTG CCT GCC CCT CAG AAC CTC TCT GTA CTC TCA	266
	Asp Glu Val Ala Ile Leu Pro Ala Pro Gln Asn Leu Ser Val Leu Ser	
	30 35 40 45	
30	ACC AAC ATG AAG CAT CTC TTG ATG TGG AGC CCA GTG ATC GCG CCT GGA	314
	Thr Asn Met Lys His Leu Leu Met Trp Ser Pro Val Ile Ala Pro Gly	
	50 55 60	
	GAA ACA GTG TAC TAT TCT GTC GAA TAC CAG GGG GAG TAC GAG AGC CTG	362
35	Glu Thr Val Tyr Tyr Ser Val Glu Tyr Gln Gly Glu Tyr Glu Ser Leu	
	65 70 75	
	TAC ACG AGC CAC ATC TGG ATC CCC AGC AGC TGG TGC TCA CTC ACT GAA	410
40	Tyr Thr Ser His Ile Trp Ile Pro Ser Ser Trp Cys Ser Leu Thr Glu	
	80 85 90	
	GGT CCT GAG TGT GAT GTC ACT GAT GAC ATC ACG GCC ACT GTG CCA TAC	458
	Gly Pro Glu Cys Asp Val Thr Asp Asp Ile Thr Ala Thr Val Pro Tyr	
	95 100 105	
45	AAC CTT CGT GTC AGG GCC ACA TTG GGC TCA CAG ACC TCA GCC TGG AGC	506
	Asn Leu Arg Val Arg Ala Thr Leu Gly Ser Gln Thr Ser Ala Trp Ser	
	110 115 120 125	
50	ATC CTG AAG CAT CCC TTT AAT AGA AAC TCA ACC ATC CTT ACC CGA CCT	554
	Ile Leu Lys His Pro Phe Asn Arg Asn Ser Thr Ile Leu Thr Arg Pro	
	130 135 140	
	GGG ATG GAG ATC CCC AAA CAT GGC TTC CAC CTG GTT ATT GAG CTG GAG	602
55	Gly Met Glu Ile Pro Lys His Gly Phe His Leu Val Ile Glu Leu Glu	
	145 150 155	

	GAC CTG GGG CCC CAG TTT GAG TTC CTT GTG GCC TAC TGG ACG AGG GAG	650
	Asp Leu Gly Pro Gln Phe Glu Phe Leu Val Ala Tyr Trp Thr Arg Glu	
	160 165 170	
5	CCT GGT GCC GAG GAA CAT GTC AAA ATG GTG AGG AGT GGG GGT ATT CCA	698
	Pro Gly Ala Glu Glu His Val Lys Met Val Arg Ser Gly Gly Ile Pro	
	175 180 185	
10	GTG CAC CTA GAA ACC ATG GAG CCA GGG GCT GCA TAC TGT GTG AAG GCC	746
	Val His Leu Glu Thr Met Glu Pro Gly Ala Ala Tyr Cys Val Lys Ala	
	190 195 200 205	
15	CAG ACA TTC GTG AAG GCC ATT GGG AGG TAC AGC GCC TTC AGC CAG ACA	794
	Gln Thr Phe Val Lys Ala Ile Gly Arg Tyr Ser Ala Phe Ser Gln Thr	
	210 215 220	
20	GAA TGT GTG GAG GTG CAA GGA GAG GCC ATT CCC CTG GTA CTG GCC CTG	842
	Glu Cys Val Glu Val Gln Gly Glu Ala Ile Pro Leu Val Leu Ala Leu	
	225 230 235	
25	TTT GCC TTT GTT GGC TTC ATG CTG ATC CTT GTG GTC GTG CCA CTG TTC	890
	Phe Ala Phe Val Gly Phe Met Leu Ile Leu Val Val Val Pro Leu Phe	
	240 245 250	
30	GTC CTC CCA GAC ACC TTG AAA ATA ACC AAT TCA CCC CAG AAG TTA ATC	938
	Val Leu Pro Asp Thr Leu Lys Ile Thr Asn Ser Pro Gln Lys Leu Ile	
	270 275 280 285	
35	AGC TGC AGA AGG GAG GAG GTG GAT GCC TGT GCC ACG GCT GTG ATG TCT	1034
	Ser Cys Arg Arg Glu Glu Val Asp Ala Cys Ala Thr Ala Val Met Ser	
	290 295 300	
40	CCT GAG GAA CTC CTC AGG GCC TGG ATC TCA TAGGTTTGCG GAAGGGCCCA	1084
	Pro Glu Glu Leu Leu Arg Ala Trp Ile Ser	
	305 310	
45	GGTGAAGCCG AGAACCTGGT CTGCATGACA TGGAACCAT GAGGGGACAA GTTGTGTTTC	1144
	TGTTTTCCGC CACGGACAAG GGATGAGAGA AGTAGGAAGA GCCTGTTGTC TACAAGTCTA	1204
	GAAGCAACCA TCAGAGGCAG GGTGGTTTGT CTAACAGAAC AACTGACTGA GGCTATGGGG	1264
	GTTGTGACCT CTAGACTTTG GGCTTCCACT TGCTTGCTG AGCAACCCTG GGAAAAGTGA	1324
	CTTCATCCCT TCGGTCCCAA GTTTTCTCAT CTGTAATGGG GGATCCCTAC AAAACTG	1381

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Table 2: Partial nucleotide and amino acid sequences of DNAX IFN Receptor Subunit like embodiments (DIRS2), originally designated HOFNY28 (SEQ ID NO: 3 and 4). Nucleotide 193 designated C, may be C or T; additional sequencing indicates that nucleotide is C.

5	C CGG GTC GAC CCA CGC GTC CGC CTG GTT TCC CCC TGG CTG ACA GTG	46
	Arg Val Asp Pro Arg Val Arg Leu Val Ser Pro Trp Leu Thr Val	
	1 5 10 15	
10	CCT TGG TTC CTG TCC TGT TGG AAT GTT ACC ATT GGG CCT CCT GAG AGC	94
	Pro Trp Phe Leu Ser Cys Trp Asn Val Thr Ile Gly Pro Pro Glu Ser	
	20 25 30	
15	ATC TGG GTG ACG CCG GGA GAA GCC TCC CTC ATC ATC AGG TTC TCC TCT	142
	Ile Trp Val Thr Pro Gly Glu Ala Ser Leu Ile Ile Arg Phe Ser Ser	
	35 40 45	
20	CCC TTC GAC GTC CCT CCC AAC CTG GGC TAT TTC CAG TAC TAT GTC CAT	190
	Pro Phe Asp Val Pro Pro Asn Leu Gly Tyr Phe Gln Tyr Tyr Val His	
	50 55 60	
25	TAC TGG GAA AAG GCG GGA ATC CAA AAG GTT AAA GGT CCT TTC AAG AGC	238
	Tyr Trp Glu Lys Ala Gly Ile Gln Lys Val Lys Gly Pro Phe Lys Ser	
	65 70 75	
30	AAC TCC ATC GTG TTG GAT GGC TTG AGA CCC TTA AGA GAA TAC TGT TTA	286
	Asn Ser Ile Val Leu Asp Gly Leu Arg Pro Leu Arg Glu Tyr Cys Leu	
	80 85 90 95	
35	CAA GTG AAG GCG CAT CTC TTT CGC ACA TCC TGC AAC ACC TCT AGG CCC	334
	Gln Val Lys Ala His Leu Phe Arg Thr Ser Cys Asn Thr Ser Arg Pro	
	100 105 110	
40	GGC CGC TTA AGC AAC ATA ACT TGC TAC GAA ACA ATG ATG GAT GCC ACT	382
	Gly Arg Leu Ser Asn Ile Thr Cys Tyr Glu Thr Met Met Asp Ala Thr	
	115 120 125	
45	ACG AAG CTT CAA CAA GTC ATC CTC ATC GCC GTG GGA GTC TTT CTG TCG	430
	Thr Lys Leu Gln Gln Val Ile Leu Ile Ala Val Gly Val Phe Leu Ser	
	130 135 140	
50	CTG GCG GCG CTG GCG GGG GGC TGT TTC TTC CTG GTG CTG AGA TAC AAA	478
	Leu Ala Ala Leu Ala Gly Gly Cys Phe Phe Leu Val Leu Arg Tyr Lys	
	145 150 155	
55	GGC CTG GTG AAA TAC TGG TTT CAC TCT CCG CCA AGC ATC CCA TCA CAA	526
	Gly Leu Val Lys Tyr Trp Phe His Ser Pro Pro Ser Ile Pro Ser Gln	
	160 165 170 175	
60	ATC GAA GAG TAT CTG AAG GAC CCG AGC CAG CCT ATC CTA GAG GCC CTG	574
	Ile Glu Glu Tyr Leu Lys Asp Pro Ser Gln Pro Ile Leu Glu Ala Leu	
	180 185 190	

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	GAC AAG GAC ACG TCA CCA ACA GAT GAT GCC TGG GAC TTG GTG TCT GTT	622
	Asp Lys Asp Thr Ser Pro Thr Asp Asp Ala Trp Asp Leu Val Ser Val	
	195 200 205	
5	GTT GCA TTT CCA GCA AAG GAG CAA GAA GAT GTT CCC CAA AGC ACT TTG	670
	Val Ala Phe Pro Ala Lys Glu Gln Glu Asp Val Pro Gln Ser Thr Leu	
	210 215 220	
10	ACC CAA AAC TCT GGT GCG GTC TGC TAGCCTGTGG GGTAAGGGCT CTGAGCCGAG	724
	Thr Gln Asn Ser Gly Ala Val Cys	
	225 230	
	GAAGCTGCTG ATGTCCATGT CAGCACTTTA TGGAATCCGG TCCTCCATTT TCCTGTCCCC	784
15	AAAAGGCCCG TCAGTGCCTG TGAAGATGTA ACGGGTCTCA TGGGGGCGAC AAGCTTATTG	844
	ATTTTTTTTCT TCAAAC TAAG AGTTTTCTAA TCATACGCGT TTTTAGAATA ATTCTACAGA	904
	TATGTCCCCG AAAGATTAAG ATTTCTCTTA AACACTAAAA AGACATGTAA TTATTTGTTA	964
20	GCAAATGGGC GTCTGGCACG CCTCTGACAC TTTTTCGTCA GCAGCCAGGA CACGAGGTCC	1024
	CCTCCTTGAT GAAGCCCCCTC GGGCAGACCA TGTCACCTGT CCCAGCCTGC CCCAAGAAGG	1084
25	GACATTAAGT GGCCCTTCTT CATATCCAAA CACCTGGCTT GAAATGTGAT TAGCCCTGTA	1144
	AATAGTTTCA CAGAGATTAA GCCTTTTTTTT CCCCCAAGTT AGGAATAAAA GACTATAATT	1204
30	AACTTTTTTAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA	1244

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Table 3: Sequence alignment of related IFN receptor family members. DR1 is a primate DIRS1 protein sequence; DR2 is a primate DIRS2 protein sequence; the IR β is the human IFN- γ receptor beta subunit (SEQ ID NO: 5), see Soh, et al. (1994) Cell 76:793-802; and CRF is the crf2-4 protein (SEQ ID NO: 6), see Lutfalla, et al. (1993) Genomics 16:366-373:

10	DR2	-----RVD	PRVRLV----	-----	-----	-----	-----
	DR1	MQTFTMVLEE	IWTSLFMWFF	YALIPCLLTD	EVAILPAPQN	LSVLSTNMKH	LLMWSPVIAP
	IRβ	-----MRPTL	LWSLLLLLLGV	FAAAAAAPPD	PLSQLPAPQH	PKIRLYNAEQ	VLSWEPVALS
	crf	-----M	AWSLGSWLGG	CLLVSALG--	---MVPPPEN	VRMNSVNFKN	ILQWESPAFA
15	DR2	-----	-----	-----	-----	-----	-----SP
	DR1	GETVYYSVEY	QGEYES--LY	TSHIWIPSSW	CSLTEGPECD	VTDDITAT--	---VPYNLRV
	IRβ	NSTRPVVYRV	QFKYTDSKWF	TADIMSIGVN	CTQITATECD	FTAASPSAGF	PMDFNVTLRL
	crf	KGNLTFTAQY	LSYR-----	-----IFQDK	CMNTTLTECD	FSSLSKYG--	----DHTLRV
20	DR2	WLTVPWFLSC	WNVTIGPPES	IWVTPGEASL	IIRFSSPFDV	PPN-----	-LGYFQYYVH
	DR1	RATLGSQTS	WSILK-HPFN	RNSTILTRPG	MEIXKXGFHL	VIELE---DL	GPQ-----
	IRβ	RAELGALHSA	WVTMPWFQHY	RNVTVGPPEN	IEVTPGEGSL	IIRFSSPFDI	ADTS-----
	crf	RAEFADEHSD	WVNIT-FCPV	DDTIIGPP-G	MQVEVLADSL	HMRFLAPKIE	NEYETWTMKN
25	DR2	YW--EKAGIQ	KVKGPFSKNS	-IVLDGLRPL	REYCLQVKAH	LFRTSCNTSR	PGRLSNITCY
	DR1	----FEFLVA	YWXREPGAEE	HVKMVRSGGI	PVHLETMEPG	AAYCVKAQT-	-FVKAIGX--
	IRβ	-TAFFCYYVH	Y--WEKGGIQ	QVKGPFRSNS	-ISLDNLKPS	RVYCLQVQAQ	LLWNKSNIFR
	crf	VYNSWTYNVQ	YW--KNGTDE	KFQITPQYDF	-EVLRLNLEPW	TTYCVQVRG-	-FLPDRNK--
30	DR2	ETMMDATTKL	QQVILIAVG	FLSLAALAGG	CFFLVLRKYG	LVKYWFHSPP	SIPSQIEEYL
	DR1	YSAFSQTECV	EVQG-EAIP	VLALFAFVG-	-FMLILVVVP	LF--VWKMG	LLQYSCCPV
	IRβ	VGHLSNISCY	ETMADASTEL	QQVILISVGT	FSLLSVLAGA	CFFLVLRKYG	LIKYWFHTPP
	crf	AGEWSEPVCE	QTTHDETVP	WMVAVILMAS	VFMVCLALLG	CFSLWLVCYK	KTKYAFSPRN
35	DR2	ETMMDATTKL	QQVILIAVG	FLSLAALAGG	CFFLVLRKYG	LVKYWFHSPP	SIPSQIEEYL
	DR1	YSAFSQTECV	EVQG-EAIP	VLALFAFVG-	-FMLILVVVP	LF--VWKMG	LLQYSCCPV
	IRβ	VGHLSNISCY	ETMADASTEL	QQVILISVGT	FSLLSVLAGA	CFFLVLRKYG	LIKYWFHTPP
	crf	AGEWSEPVCE	QTTHDETVP	WMVAVILMAS	VFMVCLALLG	CFSLWLVCYK	KTKYAFSPRN
40	DR2	KDPSQPILEA	LDKDTSP	AWDLVSVVAF	PAK--EQE--	DVPQSTLTQN	
	DR1	VLPDTLKITN	S-P-QKLISC	R----REEVD	AC--ATAVMS	PEE-----	
	IRβ	SIPLQIEEYL	KDPTQPILEA	LDKDSSPKDD	VWDSVSIISF	PEK--EQE--	
	crf	SLPQHLKEFL	GHPHNTLLF	FSFPLSDEND	VFDKLSVIAE	DSESGKQNP	
45	DR2	SGAVC					
	DR1	-LLRAWIS					
	IRβ	DVLQTL					
	crf	DSCSLGTPPG	QGPQS				

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Table 3 shows comparison of the available sequences of primate embodiments of DIRS1, DIRS2, and two related interferon receptor family members. Both of the new DIRS appear to exhibit sequence similarity to beta interferon receptor subunits.

5 Structural features of the human DIRS1, and similarly for
the other receptors as aligned in Table 3, include
characteristic transmembrane segments of the IR β and crf from
261-273, and correspond to: from about val1 to pro133;
fibronectin domains corresponding to the DIRS1 sequence from
10 about gly134 to pro232, gly233 to gly306, and pro307 to lys403;
a transmembrane segment from about val404 to gly427; and an
intracellular domain from about arg428 to the carboxy terminus.
Of particular interest is the WGEWS motif corresponding to
residues trp104 to ser108.

As used herein, the term DIRS1 shall be used to describe a protein comprising a protein or peptide segment having or sharing the amino acid sequence shown in Table 1, or a substantial fragment thereof. The invention also includes a protein variation of the respective DIRS1 allele whose sequence is provided, e.g., a mutein or soluble extracellular construct. Typically, such agonists or antagonists will exhibit less than about 10% sequence differences, and thus will often have between 1- and 11-fold substitutions, e.g., 2-, 3-, 5-, 7-fold, and others.

25 It also encompasses allelic and other variants, e.g.,
natural polymorphic, of the protein described. Typically,
it will bind to its corresponding biological ligand,
perhaps in a dimerized state with an alpha receptor
subunit, with high affinity, e.g., at least about 100 nM,
30 usually better than about 30 nM, preferably better than
about 10 nM, and more preferably at better than about 3 nM.
The term shall also be used herein to refer to related
naturally occurring forms, e.g., alleles, polymorphic
variants, and metabolic variants of the mammalian protein.

35 This invention also encompasses proteins or peptides
having substantial amino acid sequence identity with the
amino acid sequence in Table 1. It will include sequence

variants with relatively few substitutions, e.g., preferably less than about 3-5. Other embodiments include forms in association with an alpha subunit, e.g., a DSRS1, and/or with ligand, e.g., DIL-30.

5 A substantial polypeptide "fragment", or "segment", is a stretch of amino acid residues of at least about 8 amino acids, generally at least 10 amino acids, more generally at least 12 amino acids, often at least 14 amino acids, more often at least 16 amino acids, typically at least 18 amino
10 acids, more typically at least 20 amino acids, usually at least 22 amino acids, more usually at least 24 amino acids, preferably at least 26 amino acids, more preferably at least 28 amino acids, and, in particularly preferred
15 embodiments, at least about 30 or more amino acids, e.g., 35, 40, 50, 70, 90, 110, etc. Specific ends may be at all possible or appropriate combinations, or at proline residues. Sequences of segments of different proteins can be compared to one another over appropriate length stretches.

20 The invention provides polypeptides exhibiting a plurality of distinct, e.g., nonoverlapping, segments of the specified length. Typically, the plurality will be at least two, more usually at least three, and preferably 5, 7, or even more. While the length minima are provided,
25 longer lengths, of various sizes, may be appropriate, e.g., one of length 7, and two of length 12.

 Amino acid sequence homology, or sequence identity, is determined by optimizing residue matches. In some comparisons, gaps may be introduced, as required. See,
30 e.g., Needleham, et al. (1970) J. Mol. Biol. 48:443-453; Sankoff, et al., (1983) chapter one in Time Warps, String Edits, and Macromolecules: The Theory and Practice of Sequence Comparison, Addison-Wesley, Reading, MA; and software packages from NCBI, NIH; and the University of
35 Wisconsin Genetics Computer Group (GCG), Madison, WI; each of which is incorporated herein by reference. This changes when considering conservative substitutions as matches.

Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. Homologous amino acid sequences are intended to include natural allelic and interspecies variations in the cytokine sequence. Typical homologous proteins or peptides will have from 50-100% homology (if gaps can be introduced), to 60-100% homology (if conservative substitutions are included) with an amino acid sequence segment of Table 1. Homology measures will be at least about 70%, generally at least 76%, more generally at least 81%, often at least 85%, more often at least 88%, typically at least 90%, more typically at least 92%, usually at least 94%, more usually at least 95%, preferably at least 96%, and more preferably at least 97%, and in particularly preferred embodiments, at least 98% or more. The degree of homology will vary with the length of the compared segments. Homologous proteins or peptides, such as the allelic variants, will share most biological activities with the embodiments described in Table 1.

As used herein, the term "biological activity" is used to describe, without limitation, effects on inflammatory responses, innate immunity, and/or morphogenic development by cytokine-like ligands. For example, these receptors should mediate phosphatase or phosphorylase activities, which activities are easily measured by standard procedures. See, e.g., Hardie, et al. (eds. 1995) The Protein Kinase FactBook vols. I and II, Academic Press, San Diego, CA; Hanks, et al. (1991) Meth. Enzymol. 200:38-62; Hunter, et al. (1992) Cell 70:375-388; Lewin (1990) Cell 61:743-752; Pines, et al. (1991) Cold Spring Harbor Symp. Quant. Biol. 56:449-463; and Parker, et al. (1993) Nature 363:736-738. The receptors, or portions thereof, may be useful as phosphate labeling enzymes to label general or specific substrates.

The terms ligand, agonist, antagonist, and analog of, e.g., a DIRS1, include molecules that modulate the characteristic cellular responses to cytokine ligand proteins, as well as molecules possessing the more standard structural binding competition features of ligand-receptor interactions, e.g., where the receptor is a natural receptor or an antibody. The cellular responses likely are typically mediated through receptor tyrosine kinase pathways.

Also, a ligand is a molecule which serves either as a natural ligand to which said receptor, or an analog thereof, binds, or a molecule which is a functional analog of the natural ligand. The functional analog may be a ligand with structural modifications, or may be a wholly unrelated molecule which has a molecular shape which interacts with the appropriate ligand binding determinants. The ligands may serve as agonists or antagonists, see, e.g., Goodman, et al. (eds. 1990) Goodman & Gilman's: The Pharmacological Bases of Therapeutics, Pergamon Press, New York.

Rational drug design may also be based upon structural studies of the molecular shapes of a receptor or antibody and other effectors or ligands. See, e.g., Herz, et al. (1997) J. Recept. Signal Transduct. Res. 17:671-776; and Chaiken, et al. (1996) Trends Biotechnol. 14:369-375. Effectors may be other proteins which mediate other functions in response to ligand binding, or other proteins which normally interact with the receptor. One means for determining which sites interact with specific other proteins is a physical structure determination, e.g., x-ray crystallography or 2 dimensional NMR techniques. These will provide guidance as to which amino acid residues form molecular contact regions. For a detailed description of protein structural determination, see, e.g., Blundell and Johnson (1976) Protein Crystallography, Academic Press, New York, which is hereby incorporated herein by reference.

II. Activities

The cytokine receptor-like proteins will have a number of different biological activities, e.g., modulating cell proliferation, or in phosphate metabolism, being added to or removed from specific substrates, typically proteins. Such will generally result in modulation of an inflammatory function, other innate immunity response, or a morphological effect. The subunit will probably have a specific low affinity binding to the ligand.

10 The DIRS1 has the characteristic motifs of a receptor signaling through the JAK pathway. See, e.g., Ihle, et al. (1997) Stem Cells 15(suppl. 1):105-111; Silvennoinen, et al. (1997) APMIS 105:497-509; Levy (1997) Cytokine Growth Factor Review 8:81-90; Winston and Hunter (1996) Current Biol. 6:668-671; Barrett (1996) Baillieres Clin. Gastroenterol. 10:1-15; and Briscoe, et al. (1996) Philos. Trans. R. Soc. Lond. B. Biol. Sci. 351:167-171.

20 The biological activities of the cytokine receptor subunits will be related to addition or removal of phosphate moieties to substrates, typically in a specific manner, but occasionally in a non specific manner. Substrates may be identified, or conditions for enzymatic activity may be assayed by standard methods, e.g., as described in Hardie, et al. (eds. 1995) The Protein Kinase FactBook vols. I and II, Academic Press, San Diego, CA; Hanks, et al. (1991) Meth. Enzymol. 200:38-62; Hunter, et al. (1992) Cell 70:375-388; Lewin (1990) Cell 61:743-752; Pines, et al. (1991) Cold Spring Harbor Symp. Quant. Biol. 56:449-463; and Parker, et al. (1993) Nature 363:736-738.

30

III. Nucleic Acids

35 This invention contemplates use of isolated nucleic acid or fragments, e.g., which encode these or closely related proteins, or fragments thereof, e.g., to encode a corresponding polypeptide, preferably one which is biologically active. In addition, this invention covers isolated or recombinant DNAs which encode such proteins or

polypeptides having characteristic sequences of the DIRS1s. Typically, the nucleic acid is capable of hybridizing, under appropriate conditions, with a nucleic acid sequence segment shown in Table 1, but preferably not with a
5 corresponding segment of other receptors described in Table 3. Said biologically active protein or polypeptide can be a full length protein, or fragment, and will typically have a segment of amino acid sequence highly homologous, e.g., exhibiting significant stretches of identity, to one shown
10 in Table 1. Further, this invention covers the use of isolated or recombinant nucleic acid, or fragments thereof, which encode proteins having fragments which are equivalent to the DIRS1 proteins. The isolated nucleic acids can have the respective regulatory sequences in the 5' and 3'
15 flanks, e.g., promoters, enhancers, poly-A addition signals, and others from the natural gene.

An "isolated" nucleic acid is a nucleic acid, e.g., an RNA, DNA, or a mixed polymer, which is substantially pure, e.g., separated from other components which naturally
20 accompany a native sequence, such as ribosomes, polymerases, and flanking genomic sequences from the originating species. The term embraces a nucleic acid sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned
25 DNA isolates, which are thereby distinguishable from naturally occurring compositions, and chemically synthesized analogs or analogs biologically synthesized by heterologous systems. A substantially pure molecule includes isolated forms of the molecule, either completely
30 or substantially pure.

An isolated nucleic acid will generally be a homogeneous composition of molecules, but will, in some embodiments, contain heterogeneity, preferably minor. This heterogeneity is typically found at the polymer ends or
35 portions not critical to a desired biological function or activity.

A "recombinant" nucleic acid is typically defined either by its method of production or its structure. In reference to its method of production, e.g., a product made by a process, the process is use of recombinant nucleic acid techniques, e.g., involving human intervention in the nucleotide sequence. Typically this intervention involves in vitro manipulation, although under certain circumstances it may involve more classical animal breeding techniques. Alternatively, it can be a nucleic acid made by generating a sequence comprising fusion of two fragments which are not naturally contiguous to each other, but is meant to exclude products of nature, e.g., naturally occurring mutants as found in their natural state. Thus, for example, products made by transforming cells with an unnaturally occurring vector is encompassed, as are nucleic acids comprising sequence derived using any synthetic oligonucleotide process. Such a process is often done to replace a codon with a redundant codon encoding the same or a conservative amino acid, while typically introducing or removing a restriction enzyme sequence recognition site. Alternatively, the process is performed to join together nucleic acid segments of desired functions to generate a single genetic entity comprising a desired combination of functions not found in the commonly available natural forms, e.g., encoding a fusion protein. Restriction enzyme recognition sites are often the target of such artificial manipulations, but other site specific targets, e.g., promoters, DNA replication sites, regulation sequences, control sequences, or other useful features may be incorporated by design. A similar concept is intended for a recombinant, e.g., fusion, polypeptide. This will include a dimeric repeat. Specifically included are synthetic nucleic acids which, by genetic code redundancy, encode equivalent polypeptides to fragments of DIRS1 and fusions of sequences from various different related molecules, e.g., other cytokine receptor family members.

A "fragment" in a nucleic acid context is a contiguous segment of at least about 17 nucleotides, generally at least 21 nucleotides, more generally at least 25 nucleotides, ordinarily at least 30 nucleotides, more
5 ordinarily at least 35 nucleotides, often at least 39 nucleotides, more often at least 45 nucleotides, typically at least 50 nucleotides, more typically at least 55 nucleotides, usually at least 60 nucleotides, more usually at least 66 nucleotides, preferably at least 72
10 nucleotides, more preferably at least 79 nucleotides, and in particularly preferred embodiments will be at least 85 or more nucleotides. Typically, fragments of different genetic sequences can be compared to one another over appropriate length stretches, particularly defined segments
15 such as the domains described below.

A nucleic acid which codes for a DIRS1 will be particularly useful to identify genes, mRNA, and cDNA species which code for itself or closely related proteins, as well as DNAs which code for polymorphic, allelic, or
20 other genetic variants, e.g., from different individuals or related species. Preferred probes for such screens are those regions of the interleukin which are conserved between different polymorphic variants or which contain nucleotides which lack specificity, and will preferably be
25 full length or nearly so. In other situations, polymorphic variant specific sequences will be more useful.

This invention further covers recombinant nucleic acid molecules and fragments having a nucleic acid sequence identical to or highly homologous to the isolated DNA set
30 forth herein. In particular, the sequences will often be operably linked to DNA segments which control transcription, translation, and DNA replication. These additional segments typically assist in expression of the desired nucleic acid segment.

35 Homologous, or highly identical, nucleic acid sequences, when compared to one another, e.g., DIRS1 sequences, exhibit significant similarity. The standards

for homology in nucleic acids are either measures for homology generally used in the art by sequence comparison or based upon hybridization conditions. Comparative hybridization conditions are described in greater detail below.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Optical alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2:482, by the homology alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson and Lipman (1988) Proc. Nat'l Acad. Sci. USA 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (see generally Ausubel et al., supra).

One example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments to show relationship and percent sequence identity. It also plots a tree or dendrogram showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng and Doolittle (1987) J. Mol. Evol. 35:351-360. The method used is similar to the method described by Higgins and Sharp (1989) CABIOS 5:151-153. The program can align up to 300 sequences, each of a maximum length of 5,000

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nucleotides or amino acids. The multiple alignment procedure begins with the pairwise alignment of the two most similar sequences, producing a cluster of two aligned sequences. This cluster is then aligned to the next most related sequence or cluster of aligned sequences. Two clusters of sequences are aligned by a simple extension of the pairwise alignment of two individual sequences. The final alignment is achieved by a series of progressive, pairwise alignments. The program is run by designating specific sequences and their amino acid or nucleotide coordinates for regions of sequence comparison and by designating the program parameters. For example, a reference sequence can be compared to other test sequences to determine the percent sequence identity relationship using the following parameters: default gap weight (3.00), default gap length weight (0.10), and weighted end gaps.

Another example of algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described Altschul, et al. (1990) J. Mol. Biol. 215:403-410. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul, et al., supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to

the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLAST program
5 uses as defaults a wordlength (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1989) Proc. Nat'l Acad. Sci. USA 89:10915) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

10 In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin and Altschul (1993) Proc. Nat'l Acad. Sci. USA 90:5873-5787). One measure of similarity provided by the BLAST algorithm
15 is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a
20 comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

A further indication that two nucleic acid sequences of polypeptides are substantially identical is that the
25 polypeptide encoded by the first nucleic acid is immunologically cross reactive with the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides
30 differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions, as described below. Hybridization under stringent conditions should
35 give a background of at least 2-fold over background, preferably at least 3-5 or more.

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Substantial identity in the nucleic acid sequence comparison context means either that the segments, or their complementary strands, when compared, are identical when optimally aligned, with appropriate nucleotide insertions or deletions, in at least about 60% of the nucleotides, generally at least 66%, ordinarily at least 71%, often at least 76%, more often at least 80%, usually at least 84%, more usually at least 88%, typically at least 91%, more typically at least about 93%, preferably at least about 95%, more preferably at least about 96 to 98% or more, and in particular embodiments, as high at about 99% or more of the nucleotides, including, e.g., segments encoding structural domains such as the segments described below. Alternatively, substantial identity will exist when the segments will hybridize under selective hybridization conditions, to a strand or its complement, typically using a sequence derived from Table 1. Typically, selective hybridization will occur when there is at least about 55% homology over a stretch of at least about 14 nucleotides, more typically at least about 65%, preferably at least about 75%, and more preferably at least about 90%. See, Kanehisa (1984) Nucl. Acids Res. 12:203-213, which is incorporated herein by reference. The length of homology comparison, as described, may be over longer stretches, and in certain embodiments will be over a stretch of at least about 17 nucleotides, generally at least about 20 nucleotides, ordinarily at least about 24 nucleotides, usually at least about 28 nucleotides, typically at least about 32 nucleotides, more typically at least about 40 nucleotides, preferably at least about 50 nucleotides, and more preferably at least about 75 to 100 or more nucleotides. This includes, e.g., 125, 150, 175, 200, 225, 246, 273, and other lengths.

Stringent conditions, in referring to homology in the hybridization context, will be stringent combined conditions of salt, temperature, organic solvents, and other parameters typically controlled in hybridization

reactions. Stringent temperature conditions will usually include temperatures in excess of about 30° C, more usually in excess of about 37° C, typically in excess of about 45° C, more typically in excess of about 55° C, preferably in excess of about 65° C, and more preferably in excess of about 70° C. Stringent salt conditions will ordinarily be less than about 500 mM, usually less than about 400 mM, more usually less than about 300 mM, typically less than about 200 mM, preferably less than about 100 mM, and more preferably less than about 80 mM, even down to less than about 20 mM. However, the combination of parameters is much more important than the measure of any single parameter. See, e.g., Wetmur and Davidson (1968) J. Mol. Biol. 31:349-370, which is hereby incorporated herein by reference.

The isolated DNA can be readily modified by nucleotide substitutions, nucleotide deletions, nucleotide insertions, and inversions of nucleotide stretches. These modifications result in novel DNA sequences which encode this protein or its derivatives. These modified sequences can be used to produce mutant proteins (muteins) or to enhance the expression of variant species. Enhanced expression may involve gene amplification, increased transcription, increased translation, and other mechanisms. Such mutant DIRS1-like derivatives include predetermined or site-specific mutations of the protein or its fragments, including silent mutations using genetic code degeneracy. "Mutant DIRS1" as used herein encompasses a polypeptide otherwise falling within the homology definition of the DIRS1 as set forth above, but having an amino acid sequence which differs from that of other cytokine receptor-like proteins as found in nature, whether by way of deletion, substitution, or insertion. In particular, "site specific mutant DIRS1" encompasses a protein having substantial sequence identity with a protein of Table 1, and typically shares most of the biological activities or effects of the forms disclosed herein.

Although site specific mutation sites are predetermined, mutants need not be site specific. Mammalian DIRS1 mutagenesis can be achieved by making amino acid insertions or deletions in the gene, coupled with
5 expression. Substitutions, deletions, insertions, or many combinations may be generated to arrive at a final construct. Insertions include amino- or carboxy- terminal fusions. Random mutagenesis can be conducted at a target codon and the expressed mammalian DIRS1 mutants can then be
10 screened for the desired activity, providing some aspect of a structure-activity relationship. Methods for making substitution mutations at predetermined sites in DNA having a known sequence are well known in the art, e.g., by M13 primer mutagenesis. See also Sambrook, et al. (1989) and
15 Ausubel, et al. (1987 and periodic Supplements).

The mutations in the DNA normally should not place coding sequences out of reading frames and preferably will not create complementary regions that could hybridize to produce secondary mRNA structure such as loops or hairpins.

20 The phosphoramidite method described by Beaucage and Carruthers (1981) Tetra. Letts. 22:1859-1862, will produce suitable synthetic DNA fragments. A double stranded fragment will often be obtained either by synthesizing the complementary strand and annealing the strand together
25 under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

Polymerase chain reaction (PCR) techniques can often be applied in mutagenesis. Alternatively, mutagenesis
30 primers are commonly used methods for generating defined mutations at predetermined sites. See, e.g., Innis, et al. (eds. 1990) PCR Protocols: A Guide to Methods and Applications Academic Press, San Diego, CA; and Dieffenbach and Dveksler (eds. 1995) PCR Primer: A Laboratory Manual
35 Cold Spring Harbor Press, CSH, NY.

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IV. Proteins, Peptides

As described above, the present invention encompasses primate DIRS1, e.g., whose sequences are disclosed in Table 1, and described above. Allelic and other variants are
5 also contemplated, including, e.g., fusion proteins combining portions of such sequences with others, including epitope tags and functional domains.

The present invention also provides recombinant proteins, e.g., heterologous fusion proteins using segments
10 from these rodent proteins. A heterologous fusion protein is a fusion of proteins or segments which are naturally not normally fused in the same manner. Thus, the fusion product of a DIRS1 with another cytokine receptor is a continuous protein molecule having sequences fused in a
15 typical peptide linkage, typically made as a single translation product and exhibiting properties, e.g., sequence or antigenicity, derived from each source peptide. A similar concept applies to heterologous nucleic acid sequences.

20 In addition, new constructs may be made from combining similar functional or structural domains from other related proteins, e.g., cytokine receptors or Toll-like receptors, including species variants. For example, ligand-binding or other segments may be "swapped" between different new
25 fusion polypeptides or fragments. See, e.g., Cunningham, et al. (1989) Science 243:1330-1336; and O'Dowd, et al. (1988) J. Biol. Chem. 263:15985-15992, each of which is incorporated herein by reference. Thus, new chimeric polypeptides exhibiting new combinations of specificities
30 will result from the functional linkage of receptor-binding specificities. For example, the ligand binding domains from other related receptor molecules may be added or substituted for other domains of this or related proteins. The resulting protein will often have hybrid function and
35 properties. For example, a fusion protein may include a targeting domain which may serve to provide sequestering of the fusion protein to a particular subcellular organelle.

Candidate fusion partners and sequences can be selected from various sequence data bases, e.g., GenBank; NCBI, NIH; and BCG, University of Wisconsin Biotechnology Computing Group, Madison, WI, which are each incorporated
5 herein by reference.

The present invention particularly provides muteins which bind cytokine-like ligands, and/or which are affected in signal transduction. Structural alignment of human DIRS1 with other members of the cytokine receptor family
10 show conserved features/residues. See Table 3. Alignment of the human DIRS1 sequence with other members of the cytokine receptor family indicates various structural and functionally shared features. See also, Bazan, et al. (1996) Nature 379:591; Lodi, et al. (1994) Science
15 263:1762-1766; Sayle and Milner-White (1995) TIBS 20:374-376; and Gronenberg, et al. (1991) Protein Engineering 4:263-269.

Substitutions with either mouse sequences or human sequences are particularly preferred. Conversely,
20 conservative substitutions away from the ligand binding interaction regions will probably preserve most signaling activities; and conservative substitutions away from the intracellular domains will probably preserve most ligand binding properties.

"Derivatives" of the primate DIRS1 include amino acid sequence mutants, glycosylation variants, metabolic derivatives and covalent or aggregative conjugates with other chemical moieties. Covalent derivatives can be prepared by linkage of functionalities to groups which are
30 found in the DIRS1 amino acid side chains or at the N- or C- termini, e.g., by means which are well known in the art. These derivatives can include, without limitation, aliphatic esters or amides of the carboxyl terminus, or of residues containing carboxyl side chains, O-acyl
35 derivatives of hydroxyl group-containing residues, and N-acyl derivatives of the amino terminal amino acid or amino-group containing residues, e.g., lysine or arginine.

Acyl groups are selected from the group of alkyl-moieties, including C3 to C18 normal alkyl, thereby forming alkanoyl aroyl species.

In particular, glycosylation alterations are included, e.g., made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing, or in further processing steps. Particularly preferred means for accomplishing this are by exposing the polypeptide to glycosylating enzymes derived from cells which normally provide such processing, e.g., mammalian glycosylation enzymes. Deglycosylation enzymes are also contemplated. Also embraced are versions of the same primary amino acid sequence which have other minor modifications, including phosphorylated amino acid residues, e.g., phosphotyrosine, phosphoserine, or phosphothreonine.

A major group of derivatives are covalent conjugates of the receptors or fragments thereof with other proteins of polypeptides. These derivatives can be synthesized in recombinant culture such as N- or C-terminal fusions or by the use of agents known in the art for their usefulness in cross-linking proteins through reactive side groups. Preferred derivatization sites with cross-linking agents are at free amino groups, carbohydrate moieties, and cysteine residues.

Fusion polypeptides between the receptors and other homologous or heterologous proteins are also provided. Homologous polypeptides may be fusions between different receptors, resulting in, for instance, a hybrid protein exhibiting binding specificity for multiple different cytokine ligands, or a receptor which may have broadened or weakened specificity of substrate effect. Likewise, heterologous fusions may be constructed which would exhibit a combination of properties or activities of the derivative proteins. Typical examples are fusions of a reporter polypeptide, e.g., luciferase, with a segment or domain of a receptor, e.g., a ligand-binding segment, so that the presence or location of a desired ligand may be easily

determined. See, e.g., Dull, et al., U.S. Patent No. 4,859,609, which is hereby incorporated herein by reference. Other gene fusion partners include glutathione-S-transferase (GST), bacterial β -galactosidase, trpE, Protein A, β -lactamase, alpha amylase, alcohol dehydrogenase, and yeast alpha mating factor. See, e.g., Godowski, et al. (1988) Science 241:812-816.

The phosphoramidite method described by Beaucage and Carruthers (1981) Tetra. Letts. 22:1859-1862, will produce suitable synthetic DNA fragments. A double stranded fragment will often be obtained either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

Such polypeptides may also have amino acid residues which have been chemically modified by phosphorylation, sulfonation, biotinylation, or the addition or removal of other moieties, particularly those which have molecular shapes similar to phosphate groups. In some embodiments, the modifications will be useful labeling reagents, or serve as purification targets, e.g., affinity ligands.

Fusion proteins will typically be made by either recombinant nucleic acid methods or by synthetic polypeptide methods. Techniques for nucleic acid manipulation and expression are described generally, for example, in Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual (2d ed.), Vols. 1-3, Cold Spring Harbor Laboratory, and Ausubel, et al. (eds. 1987 and periodic supplements) Current Protocols in Molecular Biology, Greene/Wiley, New York, which are each incorporated herein by reference. Techniques for synthesis of polypeptides are described, for example, in Merrifield (1963) J. Amer. Chem. Soc. 85:2149-2156; Merrifield (1986) Science 232: 341-347; and Atherton, et al. (1989) Solid Phase Peptide Synthesis: A Practical Approach, IRL Press, Oxford; each of which is incorporated herein by reference. See also Dawson, et al.

(1994) Science 266:776-779 for methods to make larger polypeptides.

This invention also contemplates the use of derivatives of a DIRS1 other than variations in amino acid sequence or glycosylation. Such derivatives may involve covalent or aggregative association with chemical moieties. These derivatives generally fall into three classes: (1) salts, (2) side chain and terminal residue covalent modifications, and (3) adsorption complexes, for example with cell membranes. Such covalent or aggregative derivatives are useful as immunogens, as reagents in immunoassays, or in purification methods such as for affinity purification of a receptor or other binding molecule, e.g., an antibody. For example, a cytokine ligand can be immobilized by covalent bonding to a solid support such as cyanogen bromide-activated Sepharose, by methods which are well known in the art, or adsorbed onto polyolefin surfaces, with or without glutaraldehyde cross-linking, for use in the assay or purification of an cytokine receptor, antibodies, or other similar molecules. The ligand can also be labeled with a detectable group, for example radioiodinated by the chloramine T procedure, covalently bound to rare earth chelates, or conjugated to another fluorescent moiety for use in diagnostic assays.

An DIRS1 of this invention can be used as an immunogen for the production of antisera or antibodies specific, e.g., capable of distinguishing between other cytokine receptor family members, for the DIRS1 or various fragments thereof. The purified DIRS1 can be used to screen monoclonal antibodies or antigen-binding fragments prepared by immunization with various forms of impure preparations containing the protein. Antibodies can typically be substituted with antigen binding fragments of natural antibodies, e.g., Fab, Fab2, Fv, etc. The purified DIRS1 can also be used as a reagent to detect antibodies generated in response to the presence of elevated levels of expression, or immunological disorders which lead to

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a wide variety of cell lines or tissue samples. Natural sequences can be isolated using standard methods and the sequences provided herein, e.g., in Table 1. Other species counterparts can be identified by hybridization techniques, or by various PCR techniques, combined with or by searching in sequence databases, e.g., GenBank.

This DNA can be expressed in a wide variety of host cells for the synthesis of a full-length receptor or fragments which can in turn, for example, be used to generate polyclonal or monoclonal antibodies; for binding studies; for construction and expression of modified ligand binding or kinase/phosphatase domains; and for structure/function studies. Variants or fragments can be expressed in host cells that are transformed or transfected with appropriate expression vectors. These molecules can be substantially free of protein or cellular contaminants, other than those derived from the recombinant host, and therefore are particularly useful in pharmaceutical compositions when combined with a pharmaceutically acceptable carrier and/or diluent. The protein, or portions thereof, may be expressed as fusions with other proteins.

Expression vectors are typically self-replicating DNA or RNA constructs containing the desired receptor gene or its fragments, usually operably linked to suitable genetic control elements that are recognized in a suitable host cell. These control elements are capable of effecting expression within a suitable host. The specific type of control elements necessary to effect expression will depend upon the eventual host cell used. Generally, the genetic control elements can include a prokaryotic promoter system or a eukaryotic promoter expression control system, and typically include a transcriptional promoter, an optional operator to control the onset of transcription, transcription enhancers to elevate the level of mRNA expression, a sequence that encodes a suitable ribosome binding site, and sequences that terminate transcription

and translation. Expression vectors also usually contain an origin of replication that allows the vector to replicate independently of the host cell.

5 The vectors of this invention include those which contain DNA which encodes a protein, as described, or a fragment thereof encoding a biologically active equivalent polypeptide. The DNA can be under the control of a viral promoter and can encode a selection marker. This invention further contemplates use of such expression vectors which
10 are capable of expressing eukaryotic cDNA coding for such a protein in a prokaryotic or eukaryotic host, where the vector is compatible with the host and where the eukaryotic cDNA coding for the receptor is inserted into the vector such that growth of the host containing the vector
15 expresses the cDNA in question. Usually, expression vectors are designed for stable replication in their host cells or for amplification to greatly increase the total number of copies of the desirable gene per cell. It is not always necessary to require that an expression vector
20 replicate in a host cell, e.g., it is possible to effect transient expression of the protein or its fragments in various hosts using vectors that do not contain a replication origin that is recognized by the host cell. It is also possible to use vectors that cause integration of
25 the protein encoding portion or its fragments into the host DNA by recombination.

Vectors, as used herein, comprise plasmids, viruses, bacteriophage, integratable DNA fragments, and other vehicles which enable the integration of DNA fragments into
30 the genome of the host. Expression vectors are specialized vectors which contain genetic control elements that effect expression of operably linked genes. Plasmids are the most commonly used form of vector but all other forms of vectors which serve an equivalent function and which are, or
35 become, known in the art are suitable for use herein. See, e.g., Pouwels, et al. (1985 and Supplements) Cloning Vectors: A Laboratory Manual, Elsevier, N.Y., and

Rodriguez, et al. (eds. 1988) Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Buttersworth, Boston, which are incorporated herein by reference.

Transformed cells are cells, preferably mammalian, that have been transformed or transfected with receptor vectors constructed using recombinant DNA techniques. Transformed host cells usually express the desired protein or its fragments, but for purposes of cloning, amplifying, and manipulating its DNA, do not need to express the subject protein. This invention further contemplates culturing transformed cells in a nutrient medium, thus permitting the receptor to accumulate in the cell membrane. The protein can be recovered, either from the culture or, in certain instances, from the culture medium.

For purposes of this invention, nucleic sequences are operably linked when they are functionally related to each other. For example, DNA for a presequence or secretory leader is operably linked to a polypeptide if it is expressed as a preprotein or participates in directing the polypeptide to the cell membrane or in secretion of the polypeptide. A promoter is operably linked to a coding sequence if it controls the transcription of the polypeptide; a ribosome binding site is operably linked to a coding sequence if it is positioned to permit translation. Usually, operably linked means contiguous and in reading frame, however, certain genetic elements such as repressor genes are not contiguously linked but still bind to operator sequences that in turn control expression.

Suitable host cells include prokaryotes, lower eukaryotes, and higher eukaryotes. Prokaryotes include both gram negative and gram positive organisms, e.g., E. coli and B. subtilis. Lower eukaryotes include yeasts, e.g., S. cerevisiae and Pichia, and species of the genus Dictyostelium. Higher eukaryotes include established tissue culture cell lines from animal cells, both of non-mammalian origin, e.g., insect cells, and birds, and of mammalian origin, e.g., human, primates, and rodents.

Prokaryotic host-vector systems include a wide variety of vectors for many different species. As used herein, E. coli and its vectors will be used generically to include equivalent vectors used in other prokaryotes. A representative vector for amplifying DNA is pBR322 or many of its derivatives. Vectors that can be used to express the receptor or its fragments include, but are not limited to, such vectors as those containing the lac promoter (pUC-series); trp promoter (pBR322-trp); Ipp promoter (the pIN-series); lambda-pP or pR promoters (pOTS); or hybrid promoters such as ptac (pDR540). See Brosius, et al. (1988) "Expression Vectors Employing Lambda-, trp-, lac-, and Ipp-derived Promoters", in Vectors: A Survey of Molecular Cloning Vectors and Their Uses, (eds. Rodriguez and Denhardt), Butterworth, Boston, Chapter 10, pp. 205-236, which is incorporated herein by reference.

Lower eukaryotes, e.g., yeasts and Dictyostelium, may be transformed with DIRS1 sequence containing vectors. For purposes of this invention, the most common lower eukaryotic host is the baker's yeast, Saccharomyces cerevisiae. It will be used to generically represent lower eukaryotes although a number of other strains and species are also available. Yeast vectors typically consist of a replication origin (unless of the integrating type), a selection gene, a promoter, DNA encoding the receptor or its fragments, and sequences for translation termination, polyadenylation, and transcription termination. Suitable expression vectors for yeast include such constitutive promoters as 3-phosphoglycerate kinase and various other glycolytic enzyme gene promoters or such inducible promoters as the alcohol dehydrogenase 2 promoter or metallothionine promoter. Suitable vectors include derivatives of the following types: self-replicating low copy number (such as the YRp-series), self-replicating high copy number (such as the YEp-series); integrating types (such as the YIp-series), or mini-chromosomes (such as the YCp-series).

Higher eukaryotic tissue culture cells are normally the preferred host cells for expression of the functionally active interleukin protein. In principle, many higher eukaryotic tissue culture cell lines are workable, e.g., insect baculovirus expression systems, whether from an invertebrate or vertebrate source. However, mammalian cells are preferred. Transformation or transfection and propagation of such cells has become a routine procedure. Examples of useful cell lines include HeLa cells, Chinese hamster ovary (CHO) cell lines, baby rat kidney (BRK) cell lines, insect cell lines, bird cell lines, and monkey (COS) cell lines. Expression vectors for such cell lines usually include an origin of replication, a promoter, a translation initiation site, RNA splice sites (if genomic DNA is used), a polyadenylation site, and a transcription termination site. These vectors also usually contain a selection gene or amplification gene. Suitable expression vectors may be plasmids, viruses, or retroviruses carrying promoters derived, e.g., from such sources as from adenovirus, SV40, parvoviruses, vaccinia virus, or cytomegalovirus. Representative examples of suitable expression vectors include pCDNA1; pCD, see Okayama, et al. (1985) Mol. Cell Biol. 5:1136-1142; pMC1neo PolyA, see Thomas, et al. (1987) Cell 51:503-512; and a baculovirus vector such as pAC 373 or pAC 610.

For secreted proteins, an open reading frame usually encodes a polypeptide that consists of a mature or secreted product covalently linked at its N-terminus to a signal peptide. The signal peptide is cleaved prior to secretion of the mature, or active, polypeptide. The cleavage site can be predicted with a high degree of accuracy from empirical rules, e.g., von-Heijne (1986) Nucleic Acids Research 14:4683-4690 and Nielsen, et al. (1997) Protein Eng. 10:1-12, and the precise amino acid composition of the signal peptide often does not appear to be critical to its function, e.g., Randall, et al. (1989) Science 243:1156-1159; Kaiser et al. (1987) Science 235:312-317.

It will often be desired to express these polypeptides in a system which provides a specific or defined glycosylation pattern. In this case, the usual pattern will be that provided naturally by the expression system.

5 However, the pattern will be modifiable by exposing the polypeptide, e.g., an unglycosylated form, to appropriate glycosylating proteins introduced into a heterologous expression system. For example, the receptor gene may be co-transformed with one or more genes encoding mammalian or
10 other glycosylating enzymes. Using this approach, certain mammalian glycosylation patterns will be achievable in prokaryote or other cells.

The source of DIRS1 can be a eukaryotic or prokaryotic host expressing recombinant DIRS1, such as is described
15 above. The source can also be a cell line such as mouse Swiss 3T3 fibroblasts, but other mammalian cell lines are also contemplated by this invention, with the preferred cell line being from the human species.

Now that the sequences are known, the primate DIRS1, fragments, or derivatives thereof can be prepared by
20 conventional processes for synthesizing peptides. These include processes such as are described in Stewart and Young (1984) Solid Phase Peptide Synthesis, Pierce Chemical Co., Rockford, IL; Bodanszky and Bodanszky (1984) The Practice of Peptide Synthesis, Springer-Verlag, New York;
25 and Bodanszky (1984) The Principles of Peptide Synthesis, Springer-Verlag, New York; all of each which are incorporated herein by reference. For example, an azide process, an acid chloride process, an acid anhydride
30 process, a mixed anhydride process, an active ester process (for example, p-nitrophenyl ester, N-hydroxysuccinimide ester, or cyanomethyl ester), a carbodiimidazole process, an oxidative-reductive process, or a dicyclohexylcarbodiimide (DCCD)/additive process can be
35 used. Solid phase and solution phase syntheses are both applicable to the foregoing processes. Similar techniques can be used with partial DIRS1 sequences.

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affinity chromatography. This immunoabsorbant affinity chromatography is carried out by first linking the antibodies to a solid support and then contacting the linked antibodies with solubilized lysates of appropriate cells, lysates of other cells expressing the receptor, or lysates or supernatants of cells producing the protein as a result of DNA techniques, see below.

Generally, the purified protein will be at least about 40% pure, ordinarily at least about 50% pure, usually at least about 60% pure, typically at least about 70% pure, more typically at least about 80% pure, preferable at least about 90% pure and more preferably at least about 95% pure, and in particular embodiments, 97%-99% or more. Purity will usually be on a weight basis, but can also be on a molar basis. Different assays will be applied as appropriate.

VI. Antibodies

Antibodies can be raised to the various mammalian, e.g., primate DIRS1 proteins and fragments thereof, both in naturally occurring native forms and in their recombinant forms, the difference being that antibodies to the active receptor are more likely to recognize epitopes which are only present in the native conformations. Denatured antigen detection can also be useful in, e.g., Western analysis. Anti-idiotypic antibodies are also contemplated, which would be useful as agonists or antagonists of a natural receptor or an antibody.

Antibodies, including binding fragments and single chain versions, against predetermined fragments of the protein can be raised by immunization of animals with conjugates of the fragments with immunogenic proteins. Monoclonal antibodies are prepared from cells secreting the desired antibody. These antibodies can be screened for binding to normal or defective protein, or screened for agonistic or antagonistic activity. These monoclonal antibodies will usually bind with at least a K_D of about 1

mM, more usually at least about 300 μ M, typically at least about 100 μ M, more typically at least about 30 μ M, preferably at least about 10 μ M, and more preferably at least about 3 μ M or better.

5 The antibodies, including antigen binding fragments, of this invention can have significant diagnostic or therapeutic value. They can be potent antagonists that bind to the receptor and inhibit binding to ligand or inhibit the ability of the receptor to elicit a biological
10 response, e.g., act on its substrate. They also can be useful as non-neutralizing antibodies and can be coupled to toxins or radionuclides to bind producing cells, or cells localized to the source of the interleukin. Further, these antibodies can be conjugated to drugs or other therapeutic
15 agents, either directly or indirectly by means of a linker.

 The antibodies of this invention can also be useful in diagnostic applications. As capture or non-neutralizing antibodies, they might bind to the receptor without inhibiting ligand or substrate binding. As neutralizing
20 antibodies, they can be useful in competitive binding assays. They will also be useful in detecting or quantifying ligand. They may be used as reagents for Western blot analysis, or for immunoprecipitation or immunopurification of the respective protein.

25 Protein fragments may be joined to other materials, particularly polypeptides, as fused or covalently joined polypeptides to be used as immunogens. Mammalian cytokine receptors and fragments may be fused or covalently linked to a variety of immunogens, such as keyhole limpet
30 hemocyanin, bovine serum albumin, tetanus toxoid, etc. See Microbiology, Hoeber Medical Division, Harper and Row, 1969; Landsteiner (1962) Specificity of Serological Reactions, Dover Publications, New York; and Williams, et al. (1967) Methods in Immunology and Immunochemistry, Vol.
35 1, Academic Press, New York; each of which are incorporated herein by reference, for descriptions of methods of preparing polyclonal antisera. A typical method involves

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antibodies. Frequently, the polypeptides and antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like. Patents, teaching the use of such labels include U.S. Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant or chimeric immunoglobulins may be produced, see Cabilly, U.S. Patent No. 4,816,567; or made in transgenic mice, see, e.g., Mendez, et al. (1997) Nature Genetics 15:146-156. These references are incorporated herein by reference.

The antibodies of this invention can also be used for affinity chromatography in isolating the DIRS1 proteins or peptides. Columns can be prepared where the antibodies are linked to a solid support, e.g., particles, such as agarose, Sephadex, or the like, where a cell lysate may be passed through the column, the column washed, followed by increasing concentrations of a mild denaturant, whereby the purified protein will be released. The protein may be used to purify antibody. Conversely, the antibodies may be immunoselected or immunodepleted to provide binding compositions of defined specificities.

The antibodies may also be used to screen expression libraries for particular expression products. Usually the antibodies used in such a procedure will be labeled with a moiety allowing easy detection of presence of antigen by antibody binding.

Antibodies raised against a cytokine receptor will also be used to raise anti-idiotypic antibodies. These will be useful in detecting or diagnosing various immunological conditions related to expression of the protein or cells which express the protein. They also will

isolated using standard molecular biology and protein chemistry techniques as described herein.

Immunoassays in the competitive binding format can be used for the crossreactivity determinations. For example, the protein of SEQ ID NO: 2 can be immobilized to a solid support. Proteins added to the assay compete with the binding of the antisera to the immobilized antigen. The ability of the above proteins to compete with the binding of the antisera to the immobilized protein is compared to the proteins of IL-12 receptor beta or gp130. The percent crossreactivity for the above proteins is calculated, using standard calculations. Those antisera with less than 10% crossreactivity with each of the proteins listed above are selected and pooled. The cross-reacting antibodies are then removed from the pooled antisera by immunoabsorption with the above-listed proteins.

The immunoabsorbed and pooled antisera are then used in a competitive binding immunoassay as described above to compare a second protein to the immunogen protein (e.g., the DIRS1 like protein of SEQ ID NO: 2). In order to make this comparison, the two proteins are each assayed at a wide range of concentrations and the amount of each protein required to inhibit 50% of the binding of the antisera to the immobilized protein is determined. If the amount of the second protein required is less than twice the amount of the protein of the selected protein or proteins that is required, then the second protein is said to specifically bind to an antibody generated to the immunogen.

It is understood that these cytokine receptor proteins are members of a family of homologous proteins that comprise at least 6 so far identified genes. For a particular gene product, such as the DIRS1, the term refers not only to the amino acid sequences disclosed herein, but also to other proteins that are allelic, non-allelic, or species variants. It is also understood that the terms include nonnatural mutations introduced by deliberate mutation using conventional recombinant technology such as

single site mutation, or by excising short sections of DNA encoding the respective proteins, or by substituting new amino acids, or adding new amino acids. Such minor alterations typically will substantially maintain the immunoidentity of the original molecule and/or its biological activity. Thus, these alterations include proteins that are specifically immunoreactive with a designated naturally occurring DIRS1 protein. The biological properties of the altered proteins can be determined by expressing the protein in an appropriate cell line and measuring the appropriate effect, e.g., upon transfected lymphocytes. Particular protein modifications considered minor would include conservative substitution of amino acids with similar chemical properties, as described above for the cytokine receptor family as a whole. By aligning a protein optimally with the protein of the cytokine receptors and by using the conventional immunoassays described herein to determine immunoidentity, one can determine the protein compositions of the invention.

VII. Kits and quantitation

Both naturally occurring and recombinant forms of the cytokine receptor like molecules of this invention are particularly useful in kits and assay methods. For example, these methods would also be applied to screening for binding activity, e.g., ligands for these proteins. Several methods of automating assays have been developed in recent years so as to permit screening of tens of thousands of compounds per year. See, e.g., a BIOMEK automated workstation, Beckman Instruments, Palo Alto, California, and Fodor, et al. (1991) Science 251:767-773, which is incorporated herein by reference. The latter describes means for testing binding by a plurality of defined polymers synthesized on a solid substrate. The development of suitable assays to screen for a ligand or agonist/antagonist homologous proteins can be greatly

facilitated by the availability of large amounts of purified, soluble cytokine receptors in an active state such as is provided by this invention.

Purified DIRS1 can be coated directly onto plates for use in the aforementioned ligand screening techniques. However, non-neutralizing antibodies to these proteins can be used as capture antibodies to immobilize the respective receptor on the solid phase, useful, e.g., in diagnostic uses.

This invention also contemplates use of DIRS1, fragments thereof, peptides, and their fusion products in a variety of diagnostic kits and methods for detecting the presence of the protein or its ligand. Alternatively, or additionally, antibodies against the molecules may be incorporated into the kits and methods. Typically the kit will have a compartment containing either a DIRS1 peptide or gene segment or a reagent which recognizes one or the other. Typically, recognition reagents, in the case of peptide, would be a receptor or antibody, or in the case of a gene segment, would usually be a hybridization probe.

A preferred kit for determining the concentration of DIRS1 in a sample would typically comprise a labeled compound, e.g., ligand or antibody, having known binding affinity for DIRS1, a source of DIRS1 (naturally occurring or recombinant) as a positive control, and a means for separating the bound from free labeled compound, for example a solid phase for immobilizing the DIRS1 in the test sample. Compartments containing reagents, and instructions, will normally be provided.

Antibodies, including antigen binding fragments, specific for mammalian DIRS1 or a peptide fragment, or receptor fragments are useful in diagnostic applications to detect the presence of elevated levels of ligand and/or its fragments. Diagnostic assays may be homogeneous (without a separation step between free reagent and antibody-antigen complex) or heterogeneous (with a separation step). Various commercial assays exist, such as radioimmunoassay

receptor, or antibodies thereto can be labeled either directly or indirectly. Possibilities for direct labeling include label groups: radiolabels such as ^{125}I , enzymes (U.S. Pat. No. 3,645,090) such as peroxidase and alkaline phosphatase, and fluorescent labels (U.S. Pat. No. 3,940,475) capable of monitoring the change in fluorescence intensity, wavelength shift, or fluorescence polarization. Both of the patents are incorporated herein by reference. Possibilities for indirect labeling include biotinylation of one constituent followed by binding to avidin coupled to one of the above label groups.

There are also numerous methods of separating the bound from the free ligand, or alternatively the bound from the free test compound. The cytokine receptor can be immobilized on various matrixes followed by washing. Suitable matrices include plastic such as an ELISA plate, filters, and beads. Methods of immobilizing the receptor to a matrix include, without limitation, direct adhesion to plastic, use of a capture antibody, chemical coupling, and biotin-avidin. The last step in this approach involves the precipitation of antibody/antigen complex by any of several methods including those utilizing, e.g., an organic solvent such as polyethylene glycol or a salt such as ammonium sulfate. Other suitable separation techniques include, without limitation, the fluorescein antibody magnetizable particle method described in Rattle, et al. (1984) Clin. Chem. 30(9):1457-1461, and the double antibody magnetic particle separation as described in U.S. Pat. No. 4,659,678, each of which is incorporated herein by reference.

The methods for linking protein or fragments to various labels have been extensively reported in the literature and do not require detailed discussion here. Many of the techniques involve the use of activated carboxyl groups either through the use of carbodiimide or active esters to form peptide bonds, the formation of thioethers by reaction of a mercapto group with an

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Diagnostic kits which also test for the qualitative or quantitative presence of other markers are also contemplated. Diagnosis or prognosis may depend on the combination of multiple indications used as markers. Thus, kits may test for combinations of markers. See, e.g., Viallet, et al. (1989) Progress in Growth Factor Res. 1:89-97.

VIII. Therapeutic Utility

10 This invention provides reagents with significant therapeutic value. See, e.g., Levitzki (1996) Curr. Opin. Cell Biol. 8:239-244. The cytokine receptors (naturally occurring or recombinant), fragments thereof, mutein
15 receptors, and antibodies, along with compounds identified as having binding affinity to the receptors or antibodies, should be useful in the treatment of conditions exhibiting abnormal expression of the receptors of their ligands. Such abnormality will typically be manifested by immunological disorders. Additionally, this invention
20 should provide therapeutic value in various diseases or disorders associated with abnormal expression or abnormal triggering of response to the ligand. For example, the IL-1 ligands have been suggested to be involved in morphologic development, e.g., dorso-ventral polarity
25 determination, and immune responses, particularly the primitive innate responses. See, e.g., Sun, et al. (1991) Eur. J. Biochem. 196:247-254; and Hultmark (1994) Nature 367:116-117.

Recombinant cytokine receptors, muteins, agonist or
30 antagonist antibodies thereto, or antibodies can be purified and then administered to a patient. These reagents can be combined for therapeutic use with additional active ingredients, e.g., in conventional pharmaceutically acceptable carriers or diluents, along
35 with physiologically innocuous stabilizers and excipients. These combinations can be sterile, e.g., filtered, and placed into dosage forms as by lyophilization in dosage

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vials or storage in stabilized aqueous preparations. This invention also contemplates use of antibodies or binding fragments thereof which are not complement binding.

Ligand screening using cytokine receptor or fragments thereof can be performed to identify molecules having binding affinity to the receptors. Subsequent biological assays can then be utilized to determine if a putative ligand can provide competitive binding, which can block intrinsic stimulating activity. Receptor fragments can be used as a blocker or antagonist in that it blocks the activity of ligand. Likewise, a compound having intrinsic stimulating activity can activate the receptor and is thus an agonist in that it simulates the activity of ligand, e.g., inducing signaling. This invention further contemplates the therapeutic use of antibodies to cytokine receptors as antagonists.

The quantities of reagents necessary for effective therapy will depend upon many different factors, including means of administration, target site, reagent physiological life, pharmacological life, physiological state of the patient, and other medicants administered. Thus, treatment dosages should be titrated to optimize safety and efficacy. Typically, dosages used in vitro may provide useful guidance in the amounts useful for in situ administration of these reagents. Animal testing of effective doses for treatment of particular disorders will provide further predictive indication of human dosage. Various considerations are described, e.g., in Gilman, et al. (eds. 1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn.; each of which is hereby incorporated herein by reference. Methods for administration are discussed therein and below, e.g., for oral, intravenous, intraperitoneal, or intramuscular administration, transdermal diffusion, and others. Pharmaceutically acceptable carriers will include water, saline, buffers,

and other compounds described, e.g., in the Merck Index, Merck & Co., Rahway, New Jersey. Because of the likely high affinity binding, or turnover numbers, between a putative ligand and its receptors, low dosages of these reagents would be initially expected to be effective. And the signaling pathway suggests extremely low amounts of ligand may have effect. Thus, dosage ranges would ordinarily be expected to be in amounts lower than 1 mM concentrations, typically less than about 10 μ M concentrations, usually less than about 100 nM, preferably less than about 10 pM (picomolar), and most preferably less than about 1 fM (femtomolar), with an appropriate carrier. Slow release formulations, or slow release apparatus will often be utilized for continuous administration.

Cytokine receptors, fragments thereof, and antibodies or its fragments, antagonists, and agonists, may be administered directly to the host to be treated or, depending on the size of the compounds, it may be desirable to conjugate them to carrier proteins such as ovalbumin or serum albumin prior to their administration. Therapeutic formulations may be administered in many conventional dosage formulations. While it is possible for the active ingredient to be administered alone, it is preferable to present it as a pharmaceutical formulation. Formulations comprise at least one active ingredient, as defined above, together with one or more acceptable carriers thereof. Each carrier must be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the patient. Formulations include those suitable for oral, rectal, nasal, or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by methods well known in the art of pharmacy. See, e.g., Gilman, et al. (eds. 1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's

Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn.; Avis, et al. (eds. 1993) Pharmaceutical Dosage Forms: Parenteral Medications Dekker, NY; Lieberman, et al. (eds. 1990) Pharmaceutical Dosage Forms: Tablets

- 5 Dekker, NY; and Lieberman, et al. (eds. 1990) Pharmaceutical Dosage Forms: Disperse Systems Dekker, NY.

The therapy of this invention may be combined with or used in association with other therapeutic agents, particularly agonists or antagonists of other cytokine receptor family

10 members.

IX. Screening

- Drug screening using DIRS1 or fragments thereof can be performed to identify compounds having binding affinity to
- 15 the receptor subunit, including isolation of associated components. Subsequent biological assays can then be utilized to determine if the compound has intrinsic stimulating activity and is therefore a blocker or antagonist in that it blocks the activity of the ligand.
- 20 Likewise, a compound having intrinsic stimulating activity can activate the receptor and is thus an agonist in that it simulates the activity of a cytokine ligand. This invention further contemplates the therapeutic use of antibodies to the receptor as cytokine agonists or
- 25 antagonists.

- One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant DNA molecules expressing the DIRS1. Cells may be isolated which express a receptor in isolation from
- 30 other functional receptors. Such cells, either in viable or fixed form, can be used for standard ligand/receptor binding assays. See also, Parce, et al. (1989) Science 246:243-247; and Owicki, et al. (1990) Proc. Nat'l Acad. Sci. USA 87:4007-4011, which describe sensitive methods to
- 35 detect cellular responses. Competitive assays are particularly useful, where the cells (source of putative ligand) are contacted and incubated with a labeled receptor

or antibody having known binding affinity to the ligand, such as ^{125}I -antibody, and a test sample whose binding affinity to the binding composition is being measured. The bound and free labeled binding compositions are then

5 separated to assess the degree of ligand binding. The amount of test compound bound is inversely proportional to the amount of labeled receptor binding to the known source. Any one of numerous techniques can be used to separate bound from free ligand to assess the degree of ligand

10 binding. This separation step could typically involve a procedure such as adhesion to filters followed by washing, adhesion to plastic followed by washing, or centrifugation of the cell membranes. Viable cells could also be used to screen for the effects of drugs on cytokine mediated

15 functions, e.g., second messenger levels, i.e., Ca^{++} ; cell proliferation; inositol phosphate pool changes; and others. Some detection methods allow for elimination of a separation step, e.g., a proximity sensitive detection system. Calcium sensitive dyes will be useful for

20 detecting Ca^{++} levels, with a fluorimeter or a fluorescence cell sorting apparatus.

X. Ligands

The descriptions of the DIRS1 herein provide means to

25 identify ligands, as described above. Such ligand should bind specifically to the respective receptor with reasonably high affinity. Various constructs are made available which allow either labeling of the receptor to detect its ligand. For example, directly labeling cytokine

30 receptor, fusing onto it markers for secondary labeling, e.g., FLAG or other epitope tags, etc., will allow detection of receptor. This can be histological, as an affinity method for biochemical purification, or labeling or selection in an expression cloning approach. A two-

35 hybrid selection system may also be applied making appropriate constructs with the available cytokine receptor

sequences. See, e.g., Fields and Song (1989) Nature 340:245-246.

Generally, descriptions of cytokine receptors will be analogously applicable to individual specific embodiments
5 directed to DIRS1 reagents and compositions.

The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the inventions to the specific embodiments.

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EXAMPLES

I. General Methods

Some of the standard methods are described or
5 referenced, e.g., in Maniatis, et al. (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor Press; Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual, (2d ed.), vols. 1-3, CSH Press, NY; Ausubel, et al., Biology, Greene
10 Publishing Associates, Brooklyn, NY; or Ausubel, et al. (1987 and Supplements) Current Protocols in Molecular Biology, Greene/Wiley, New York. Methods for protein purification include such methods as ammonium sulfate precipitation, column chromatography, electrophoresis,
15 centrifugation, crystallization, and others. See, e.g., Ausubel, et al. (1987 and periodic supplements); Coligan, et al. (ed. 1996) and periodic supplements, Current Protocols In Protein Science Greene/Wiley, New York; Deutscher (1990) "Guide to Protein Purification" in Methods
20 in Enzymology, vol. 182, and other volumes in this series; and manufacturer's literature on use of protein purification products, e.g., Pharmacia, Piscataway, N.J., or Bio-Rad, Richmond, CA. Combination with recombinant techniques allow fusion to appropriate segments, e.g., to a
25 FLAG sequence or an equivalent which can be fused via a protease-removable sequence. See, e.g., Hochuli (1989) Chemische Industrie 12:69-70; Hochuli (1990) "Purification of Recombinant Proteins with Metal Chelate Absorbent" in Setlow (ed.) Genetic Engineering, Principle and Methods
30 12:87-98, Plenum Press, N.Y.; and Crowe, et al. (1992) OIAexpress: The High Level Expression & Protein Purification System QUIAGEN, Inc., Chatsworth, CA.

Computer sequence analysis is performed, e.g., using
available software programs, including those from the GCG
35 (U. Wisconsin) and GenBank sources. Public sequence databases were also used, e.g., from GenBank and others.

Many techniques applicable to IL-10 or IL-12 receptors may be applied to the DIRS1, as described, e.g., in USSN 08/110,683 (IL-10 receptor), which is incorporated herein by reference.

5

II. Computational Analysis

Human sequences related to cytokine receptors were identified from genomic sequence database using, e.g., the BLAST server (Altschul, et al. (1994) Nature Genet. 6:119-129). Standard analysis programs may be used to evaluate structure, e.g., PHD (Rost and Sander (1994) Proteins 19:55-72) and DSC (King and Sternberg (1996) Protein Sci. 5:2298-2310). Standard comparison software includes, e.g., Altschul, et al. (1990) J. Mol. Biol. 215:403-10; Waterman (1995) Introduction to Computational Biology: Maps, Sequences, and Genomes Chapman & Hall; Lander and Waterman (eds. 1995) Calculating the Secrets of Life: Applications of the Mathematical Sciences in Molecular Biology National Academy Press; and Speed and Waterman (eds. 1996) Genetic Mapping and DNA Sequencing (Ima Volumes in Mathematics and Its Applications, Vol 81) Springer Verlag.

III. Cloning of full-length DIRS cDNAs; Chromosomal localization

PCR primers derived from the DIRS sequences are used to probe a human cDNA library. Full length cDNAs for primate, rodent, or other species DIRS1 are cloned, e.g., by DNA hybridization screening of λ gt10 phage. PCR reactions are conducted using T. aquaticus Taqplus DNA polymerase (Stratagene) under appropriate conditions.

Chromosome spreads are prepared. In situ hybridization is performed on chromosome preparations obtained from phytohemagglutinin-stimulated human lymphocytes cultured for 72 h. 5-bromodeoxyuridine was added for the final seven hours of culture (60 μ g/ml of medium), to ensure a posthybridization chromosomal banding of good quality.

A PCR fragment, amplified with the help of primers, is cloned into an appropriate vector. The vector is labeled by nick-translation with ^3H . The radiolabeled probe is hybridized to metaphase spreads at final concentration of
5 200 ng/ml of hybridization solution as described in Mattei, et al. (1985) Hum. Genet. 69:327-331.

After coating with nuclear track emulsion (KODAK NTB2), slides are exposed. To avoid any slipping of silver grains during the banding procedure, chromosome spreads are
10 first stained with buffered Giemsa solution and metaphase photographed. R-banding is then performed by the fluorochrome-photolysis-Giemsa (FPG) method and metaphases rephotographed before analysis. Alternatively, mapped sequence tags may be searched in a database.

15 Similar appropriate methods are used for other species.

IV. Localization of DIRS1 or DIRS2 mRNA

Human multiple tissue (Cat# 1, 2) and cancer cell line
20 blots (Cat# 7757-1), containing approximately 2 μg of poly(A)⁺ RNA per lane, are purchased from Clontech (Palo Alto, CA). Probes are radiolabeled with [α - ^{32}P] dATP, e.g., using the Amersham Rediprime random primer labeling kit (RPN1633). Prehybridization and hybridizations are
25 performed at 65° C in 0.5 M Na₂HPO₄, 7% SDS, 0.5 M EDTA (pH 8.0). High stringency washes are conducted, e.g., at 65° C with two initial washes in 2 x SSC, 0.1% SDS for 40 min followed by a subsequent wash in 0.1 x SSC, 0.1% SDS for 20 min. Membranes are then exposed at -70° C to X-Ray film
30 (Kodak) in the presence of intensifying screens. More detailed studies by cDNA library Southernns are performed with selected human DIRS1 clones to examine their expression in hemopoietic or other cell subsets.

Alternatively, two appropriate primers are selected
35 from Table 1 or 2. RT-PCR is used on an appropriate mRNA sample selected for the presence of message to produce a cDNA, e.g., a sample which expresses the gene.

Full length clones may be isolated by hybridization of cDNA libraries from appropriate tissues pre-selected by PCR signal. Northern blots can be performed.

Message for genes encoding DIRS1 will be assayed by appropriate technology, e.g., PCR, immunoassay, hybridization, or otherwise. Tissue and organ cDNA preparations are available, e.g., from Clontech, Mountain View, CA. Identification of sources of natural expression are useful, as described. And the identification of functional receptor subunit pairings will allow for prediction of what cells express the combination of receptor subunits which will result in a physiological responsiveness to each of the cytokine ligands.

For mouse distribution, e.g., Southern Analysis can be performed: DNA (5 µg) from a primary amplified cDNA library was digested with appropriate restriction enzymes to release the inserts, run on a 1% agarose gel and transferred to a nylon membrane (Schleicher and Schuell, Keene, NH).

Samples for mouse mRNA isolation may include: resting mouse fibroblastic L cell line (C200); Braf:ER (Braf fusion to estrogen receptor) transfected cells, control (C201); T cells, TH1 polarized (Mell14 bright, CD4+ cells from spleen, polarized for 7 days with IFN-γ and anti IL-4; T200); T cells, TH2 polarized (Mell14 bright, CD4+ cells from spleen, polarized for 7 days with IL-4 and anti-IFN-γ; T201); T cells, highly TH1 polarized (see Openshaw, et al. (1995) J. Exp. Med. 182:1357-1367; activated with anti-CD3 for 2, 6, 16 h pooled; T202); T cells, highly TH2 polarized (see Openshaw, et al. (1995) J. Exp. Med. 182:1357-1367; activated with anti-CD3 for 2, 6, 16 h pooled; T203); CD44-CD25+ pre T cells, sorted from thymus (T204); TH1 T cell clone D1.1, resting for 3 weeks after last stimulation with antigen (T205); TH1 T cell clone D1.1, 10 µg/ml ConA stimulated 15 h (T206); TH2 T cell clone CDC35, resting for 3 weeks after last stimulation with antigen (T207); TH2 T cell clone CDC35, 10 µg/ml ConA stimulated 15 h (T208); Mell14+ naive T cells from spleen, resting (T209); Mell14+ T

- cells, polarized to Th1 with IFN- γ /IL-12/anti-IL-4 for 6, 12, 24 h pooled (T210); Mel14+ T cells, polarized to Th2 with IL-4/anti-IFN- γ for 6, 13, 24 h pooled (T211); unstimulated mature B cell leukemia cell line A20 (B200);
- 5 unstimulated B cell line CH12 (B201); unstimulated large B cells from spleen (B202); B cells from total spleen, LPS activated (B203); metrizamide enriched dendritic cells from spleen, resting (D200); dendritic cells from bone marrow, resting (D201); monocyte cell line RAW 264.7 activated with
- 10 LPS 4 h (M200); bone-marrow macrophages derived with GM and M-CSF (M201); macrophage cell line J774, resting (M202); macrophage cell line J774 + LPS + anti-IL-10 at 0.5, 1, 3, 6, 12 h pooled (M203); macrophage cell line J774 + LPS + IL-10 at 0.5, 1, 3, 5, 12 h pooled (M204); aerosol
- 15 challenged mouse lung tissue, Th2 primers, aerosol OVA challenge 7, 14, 23 h pooled (see Garlisi, et al. (1995) Clinical Immunology and Immunopathology 75:75-83; X206); Nippostrongylus-infected lung tissue (see Coffman, et al. (1989) Science 245:308-310; X200); total adult lung, normal
- 20 (O200); total lung, rag-1 (see Schwarz, et al. (1993) Immunodeficiency 4:249-252; O205); IL-10 K.O. spleen (see Kuhn, et al. (1991) Cell 75:263-274; X201); total adult spleen, normal (O201); total spleen, rag-1 (O207); IL-10 K.O. Peyer's patches (O202); total Peyer's patches, normal
- 25 (O210); IL-10 K.O. mesenteric lymph nodes (X203); total mesenteric lymph nodes, normal (O211); IL-10 K.O. colon (X203); total colon, normal (O212); NOD mouse pancreas (see Makino, et al. (1980) Jikken Dobutsu 29:1-13; X205); total thymus, rag-1 (O208); total kidney, rag-1 (O209); total
- 30 heart, rag-1 (O202); total brain, rag-1 (O203); total testes, rag-1 (O204); total liver, rag-1 (O206); rat normal joint tissue (O300); and rat arthritic joint tissue (X300).

Samples for human mRNA isolation may include:

- peripheral blood mononuclear cells (monocytes, T cells, NK
- 35 cells, granulocytes, B cells), resting (T100); peripheral blood mononuclear cells, activated with anti-CD3 for 2, 6, 12 h pooled (T101); T cell, TH0 clone Mot 72, resting

(T102); T cell, TH0 clone Mot 72, activated with anti-CD28 and anti-CD3 for 3, 6, 12 h pooled (T103); T cell, TH0 clone Mot 72, anergic treated with specific peptide for 2, 7, 12 h pooled (T104); T cell, TH1 clone HY06, resting (T107); T cell, TH1 clone HY06, activated with anti-CD28 and anti-CD3 for 3, 6, 12 h pooled (T108); T cell, TH1 clone HY06, anergic treated with specific peptide for 2, 6, 12 h pooled (T109); T cell, TH2 clone HY935, resting (T110); T cell, TH2 clone HY935, activated with anti-CD28 and anti-CD3 for 2, 7, 12 h pooled (T111); T cells CD4+CD45RO- T cells polarized 27 days in anti-CD28, IL-4, and anti IFN- γ , TH2 polarized, activated with anti-CD3 and anti-CD28 4 h (T116); T cell tumor lines Jurkat and Hut78, resting (T117); T cell clones, pooled AD130.2, Tc783.12, Tc783.13, Tc783.58, Tc782.69, resting (T118); T cell random $\gamma\delta$ T cell clones, resting (T119); Splenocytes, resting (B100); Splenocytes, activated with anti-CD40 and IL-4 (B101); B cell EBV lines pooled WT49, RSB, JY, CVIR, 721.221, RM3, HSY, resting (B102); B cell line JY, activated with PMA and ionomycin for 1, 6 h pooled (B103); NK 20 clones pooled, resting (K100); NK 20 clones pooled, activated with PMA and ionomycin for 6 h (K101); NKL clone, derived from peripheral blood of LGL leukemia patient, IL-2 treated (K106); NK cytotoxic clone 640-A30-1, resting (K107); hematopoietic precursor line TF1, activated with PMA and ionomycin for 1, 6 h pooled (C100); U937 premonocytic line, resting (M100); U937 premonocytic line, activated with PMA and ionomycin for 1, 6 h pooled (M101); elutriated monocytes, activated with LPS, IFN γ , anti-IL-10 for 1, 2, 6, 12, 24 h pooled (M102); elutriated monocytes, activated with LPS, IFN γ , IL-10 for 1, 2, 6, 12, 24 h pooled (M103); elutriated monocytes, activated with LPS, IFN γ , anti-IL-10 for 4, 16 h pooled (M106); elutriated monocytes, activated with LPS, IFN γ , IL-10 for 4, 16 h pooled (M107); elutriated monocytes, activated LPS for 1 h (M108); elutriated monocytes, activated LPS for 6 h (M109); DC 70% CD1a+, from CD34+ GM-CSF, TNF α 12 days, resting (D101); DC

70% CD1a+, from CD34+ GM-CSF, TNF α 12 days, activated with PMA and ionomycin for 1 hr (D102); DC 70% CD1a+, from CD34+ GM-CSF, TNF α 12 days, activated with PMA and ionomycin for 6 hr (D103); DC 95% CD1a+, from CD34+ GM-CSF, TNF α 12 days

5 FACS sorted, activated with PMA and ionomycin for 1, 6 h pooled (D104); DC 95% CD14+, ex CD34+ GM-CSF, TNF α 12 days FACS sorted, activated with PMA and ionomycin 1, 6 hr pooled (D105); DC CD1a+ CD86+, from CD34+ GM-CSF, TNF α 12 days FACS sorted, activated with PMA and ionomycin for 1, 6

10 h pooled (D106); DC from monocytes GM-CSF, IL-4 5 days, resting (D107); DC from monocytes GM-CSF, IL-4 5 days, resting (D108); DC from monocytes GM-CSF, IL-4 5 days, activated LPS 4, 16 h pooled (D109); DC from monocytes GM-CSF, IL-4 5 days, activated TNF α , monocyte supe for 4, 16 h

15 pooled (D110); leiomyoma L11 benign tumor (X101); normal myometrium M5 (O115); malignant leiomyosarcoma GS1 (X103); lung fibroblast sarcoma line MRC5, activated with PMA and ionomycin for 1, 6 h pooled (C101); kidney epithelial carcinoma cell line CHA, activated with PMA and ionomycin

20 for 1, 6 h pooled (C102); kidney fetal 28 wk male (O100); lung fetal 28 wk male (O101); liver fetal 28 wk male (O102); heart fetal 28 wk male (O103); brain fetal 28 wk male (O104); gallbladder fetal 28 wk male (O106); small intestine fetal 28 wk male (O107); adipose tissue fetal 28

25 wk male (O108); ovary fetal 25 wk female (O109); uterus fetal 25 wk female (O110); testes fetal 28 wk male (O111); spleen fetal 28 wk male (O112); adult placenta 28 wk (O113); and tonsil inflamed, from 12 year old (X100).

With a cDNA Southern, the human DIRS1 was found in

30 LPS activated dendritic cells ("DC LPS"); monokine activated dendritic cells ("DC mix"); normal skin; Psoriasis skin; inflamed tonsil; fetal liver; fetal small intestine; fetal ovary; resting "70% dendritic cells"; 6 hr activated 70% dendritic cells; and LPS activated monocytes.

35 A signal was also detected in normal monkey lung and Ascaris-challenged monkey lung (24 h), which indicates

cross species hybridization. The following libraries had weaker expression of DIRS1: smoker lung pool; fetal spleen CD4+ T cells (TH2 polarized); gamma delta T cells; activated splenocytes; and B cells.

- 5 HOFNy28 (DIRS2) is expressed in U937 (a premonocytic cell line) cells, both resting and activated; activated A549 cells (epithelial cells, IL-1 β activated); fetal uterus; fetal testes; and fetal spleen. This data is from PCR on these cDNA libraries followed by Southern
10 hybridization.

Similar samples may isolated in other species for evaluation.

V. Cloning of species counterparts of DIRS1 or DIRS2

- 15 Various strategies are used to obtain species counterparts of, e.g., the DIRS1, preferably from other primates or rodents. One method is by cross hybridization using closely related species DNA probes. It may be useful to go into evolutionarily similar species as intermediate
20 steps. Another method is by using specific PCR primers based on the identification of blocks of similarity or difference between genes, e.g., areas of highly conserved or nonconserved polypeptide or nucleotide sequence. Database sequence searches may also identify species
25 counterparts.

VI. Production of mammalian DIRS1 or DIRS2 protein

- An appropriate, e.g., GST, fusion construct is engineered for expression, e.g., in E. coli. For example,
30 a mouse IGIF pGex plasmid is constructed and transformed into E. coli. Freshly transformed cells are grown, e.g., in LB medium containing 50 μ g/ml ampicillin and induced with IPTG (Sigma, St. Louis, MO). After overnight induction, the bacteria are harvested and the pellets
35 containing the DIRS1 protein are isolated. The pellets are homogenized, e.g., in TE buffer (50 mM Tris-base pH 8.0, 10 mM EDTA and 2 mM pefabloc) in 2 liters. This material is

passed through a microfluidizer (Microfluidics, Newton, MA) three times. The fluidized supernatant is spun down on a Sorvall GS-3 rotor for 1 h at 13,000 rpm. The resulting supernatant containing the cytokine receptor protein is
5 filtered and passed over a glutathione-SEPHAROSE column equilibrated in 50 mM Tris-base pH 8.0. The fractions containing the DIRS1-GST fusion protein are pooled and cleaved, e.g., with thrombin (Enzyme Research Laboratories, Inc., South Bend, IN). The cleaved pool is then passed
10 over a Q-SEPHAROSE column equilibrated in 50 mM Tris-base. Fractions containing DIRS1 are pooled and diluted in cold distilled H₂O, to lower the conductivity, and passed back over a fresh Q-Sepharose column, alone or in succession with an immunoaffinity antibody column. Fractions
15 containing the DIRS1 protein are pooled, aliquoted, and stored in the -70° C freezer.

Comparison of the CD spectrum with cytokine receptor protein may suggest that the protein is correctly folded. See Hazuda, et al. (1969) J. Biol. Chem. 264:1689-1693.

20

VII. Determining physiological forms of receptors

The cellular forms of receptors for ligands can be tested with the various ligands and receptor subunits provided, e.g., IL-10 related sequences. In particular,
25 multiple cytokine receptor like ligands have been identified, see, e.g., USSN 60/027,368, 08/934,959, and 08/842,659, which are incorporated herein by reference..

Cotransformation of the DIRS1 with putative other receptor subunit genes may be performed. In particular,
30 the DSRS1 is suggested to be a second receptor subunit needed for functional receptor signaling. Such cells may be used to screen putative cytokine ligands, such as the DIL-30, for signaling. A cell proliferation assay may be used.

35 In addition, it has been known that many cytokine receptors function as heterodimers. The IL-1 α and IL-1 β ligands bind an IL-1R1 as the primary receptor and this

complex then forms a high affinity receptor complex with the IL-1R3. As indicated above, the sequence similarity to IL-12 receptor subunits suggests functional similarity of the functional receptor, e.g., a soluble alpha subunit, and
5 transmembrane beta subunit.

These subunit combinations can be tested now with the provided reagents. In particular, appropriate constructs can be made for transformation or transfection of subunits into cells. Constructs for the alpha chains, e.g., DSRS1
10 forms, can be made. Likewise for the beta subunit DIRS1. Combinatorial transfections of transformations can make cells expressing defined subunits, which can be tested for response to the predicted ligands. Appropriate cell types can be used, e.g., 293 T cells, with, e.g., an NFkB
15 reporter construct.

Biological assays will generally be directed to the ligand binding feature of the protein or to the kinase/phosphatase activity of the receptor. The activity will typically be reversible, as are many other enzyme
20 reactions, and may mediate phosphatase or phosphorylase activities, which activities are easily measured by standard procedures. See, e.g., Hardie, et al. (eds. 1995) The Protein Kinase FactBook vols. I and II, Academic Press, San Diego, CA; Hanks, et al. (1991) Meth. Enzymol. 200:38-
25 62; Hunter, et al. (1992) Cell 70:375-388; Lewin (1990) Cell 61:743-752; Pines, et al. (1991) Cold Spring Harbor Symp. Quant. Biol. 56:449-463; and Parker, et al. (1993) Nature 363:736-738.

The family of cytokines contains molecules which are
30 important mediators of hematopoiesis or inflammatory disease. See, e.g., Thomson (ed. 1994) The Cytokine Handbook Academic Press, San Diego; and Dinarello (1996) Blood 87:2095-2147.

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VIII. Antibodies specific for DIRS1 or DIRS2

Inbred Balb/c mice are immunized intraperitoneally with recombinant forms of the protein, e.g., purified DIRS1 or stable transfected NIH-3T3 cells. Animals are boosted at appropriate time points with protein, with or without additional adjuvant, to further stimulate antibody production. Serum is collected, or hybridomas produced with harvested spleens.

Alternatively, Balb/c mice are immunized with cells transformed with the gene or fragments thereof, either endogenous or exogenous cells, or with isolated membranes enriched for expression of the antigen. Serum is collected at the appropriate time, typically after numerous further administrations. Various gene therapy techniques may be useful, e.g., in producing protein in situ, for generating an immune response. Serum may be immunoselected or depleted to prepare substantially purified antibodies of defined specificity and high affinity. Preparations which specifically bind particular segments or defined epitopes may be made.

Monoclonal antibodies may be made. For example, splenocytes are fused with an appropriate fusion partner and hybridomas are selected in growth medium by standard procedures. Hybridoma supernatants are screened for the presence of antibodies which bind to the DIRS1, e.g., by ELISA or other assay. Antibodies which specifically recognize specific DIRS1 embodiments may also be selected or prepared.

In another method, synthetic peptides or purified protein are presented to an immune system to generate monoclonal or polyclonal antibodies. See, e.g., Coligan (ed. 1991) Current Protocols in Immunology Wiley/Greene; and Harlow and Lane (1989) Antibodies: A Laboratory Manual Cold Spring Harbor Press. In appropriate situations, the binding reagent is either labeled as described above, e.g., fluorescence or otherwise, or immobilized to a substrate for panning methods. Nucleic acids may also be introduced

into cells in an animal to produce the antigen, which serves to elicit an immune response. See, e.g., Wang, et al. (1993) Proc. Nat'l. Acad. Sci. 90:4156-4160; Barry, et al. (1994) BioTechniques 16:616-619; and Xiang, et al.

5 (1995) Immunity 2: 129-135.

Moreover, antibodies which may be useful to determine the combination of the DIRS1 with a functional alpha subunit may be generated. Thus, e.g., epitopes characteristic of a particular functional alpha/beta
10 combination may be identified with appropriate antibodies.

IX. Production of fusion proteins with DIRS1 or DIRS2

Various fusion constructs are made with DIRS1 or DIRS2. A portion of the appropriate gene is fused to an
15 epitope tag, e.g., a FLAG tag, or to a two hybrid system construct. See, e.g., Fields and Song (1989) Nature 340:245-246.

The epitope tag may be used in an expression cloning procedure with detection with anti-FLAG antibodies to
20 detect a binding partner, e.g., ligand for the respective cytokine receptor. The two hybrid system may also be used to isolate proteins which specifically bind to DIRS1.

X. Structure activity relationship

25 Information on the criticality of particular residues is determined using standard procedures and analysis. Standard mutagenesis analysis is performed, e.g., by generating many different variants at determined positions, e.g., at the positions identified above, and evaluating
30 biological activities of the variants. This may be performed to the extent of determining positions which modify activity, or to focus on specific positions to determine the residues which can be substituted to either retain, block, or modulate biological activity.

35 Alternatively, analysis of natural variants can indicate what positions tolerate natural mutations. This may result from populational analysis of variation among

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individuals, or across strains or species. Samples from selected individuals are analyzed, e.g., by PCR analysis and sequencing. This allows evaluation of population polymorphisms.

5

XI. Isolation of a ligand for DIRS1 or DIRS2

A cytokine receptor can be used as a specific binding reagent to identify its binding partner, by taking advantage of its specificity of binding, much like an antibody would be used. Typically, the binding receptor is a heterodimer of receptor subunits. A binding reagent is either labeled as described above, e.g., fluorescence or otherwise, or immobilized to a substrate for panning methods.

15 The binding composition is used to screen an expression library made from a cell line which expresses a binding partner, i.e., ligand, preferably membrane associated. Standard staining techniques are used to detect or sort surface expressed ligand, or surface expressing transformed cells are screened by panning. Screening of intracellular expression is performed by various staining or immunofluorescence procedures. See also McMahan, et al. (1991) EMBO J. 10:2821-2832.

For example, on day 0, precoat 2-chamber permanox slides with 1 ml per chamber of fibronectin, 10 ng/ml in PBS, for 30 min at room temperature. Rinse once with PBS. Then plate COS cells at $2-3 \times 10^5$ cells per chamber in 1.5 ml of growth media. Incubate overnight at 37° C.

On day 1 for each sample, prepare 0.5 ml of a solution of 66 µg/ml DEAE-dextran, 66 µM chloroquine, and 4 µg DNA in serum free DME. For each set, a positive control is prepared, e.g., of DIRS1-FLAG cDNA at 1 and 1/200 dilution, and a negative mock. Rinse cells with serum free DME. Add the DNA solution and incubate 5 hr at 37° C. Remove the medium and add 0.5 ml 10% DMSO in DME for 2.5 min. Remove and wash once with DME. Add 1.5 ml growth medium and incubate overnight.

On day 2, change the medium. On days 3 or 4, the cells are fixed and stained. Rinse the cells twice with Hank's Buffered Saline Solution (HBSS) and fix in 4% paraformaldehyde (PFA)/glucose for 5 min. Wash 3X with
5 HBSS. The slides may be stored at -80° C after all liquid is removed. For each chamber, 0.5 ml incubations are performed as follows. Add HBSS/saponin (0.1%) with 32 µl/ml of 1 M NaN₃ for 20 min. Cells are then washed with HBSS/saponin 1X. Add appropriate DIRS1 or DIRS1/antibody
10 complex to cells and incubate for 30 min. Wash cells twice with HBSS/saponin. If appropriate, add first antibody for 30 min. Add second antibody, e.g., Vector anti-mouse antibody, at 1/200 dilution, and incubate for 30 min. Prepare ELISA solution, e.g., Vector Elite ABC horseradish
15 peroxidase solution, and preincubate for 30 min. Use, e.g., 1 drop of solution A (avidin) and 1 drop solution B (biotin) per 2.5 ml HBSS/saponin. Wash cells twice with HBSS/saponin. Add ABC HRP solution and incubate for 30 min. Wash cells twice with HBSS, second wash for 2 min,
20 which closes cells. Then add Vector diaminobenzoic acid (DAB) for 5 to 10 min. Use 2 drops of buffer plus 4 drops DAB plus 2 drops of H₂O₂ per 5 ml of glass distilled water. Carefully remove chamber and rinse slide in water. Air dry for a few minutes, then add 1 drop of Crystal Mount and a
25 cover slip. Bake for 5 min at 85-90° C.

Evaluate positive staining of pools and progressively subclone to isolation of single genes responsible for the binding.

Alternatively, receptor reagents are used to affinity
30 purify or sort out cells expressing a putative ligand. See, e.g., Sambrook, et al. or Ausubel, et al.

Another strategy is to screen for a membrane bound receptor by panning. The receptor cDNA is constructed as described above. The ligand can be immobilized and used to
35 immobilize expressing cells. Immobilization may be achieved by use of appropriate antibodies which recognize, e.g., a FLAG sequence of a DIRS1 fusion construct, or by

use of antibodies raised against the first antibodies. Recursive cycles of selection and amplification lead to enrichment of appropriate clones and eventual isolation of receptor expressing clones.

5 Phage expression libraries can be screened by mammalian DIRS1. Appropriate label techniques, e.g., anti-FLAG antibodies, will allow specific labeling of appropriate clones.

10 All citations herein are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

15 Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited by the terms of the appended claims, along with the full scope of equivalents to which such claims
20 are entitled; and the invention is not to be limited by the specific embodiments that have been presented herein by way of example.

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WHAT IS CLAIMED IS:

1. A composition of matter selected from:
- 5 a) a substantially pure or recombinant DIRS1 polypeptide comprising at least three distinct nonoverlapping segments of at least four amino acids identical to segments of SEQ ID NO: 2;
- 10 b) a substantially pure or recombinant DIRS1 polypeptide comprising at least two distinct nonoverlapping segments of at least five amino acids identical to segments of SEQ ID NO: 2;
- c) a natural sequence DIRS1 comprising mature SEQ ID NO: 2;
- 15 d) a fusion polypeptide comprising DIRS1 sequence;
- e) a substantially pure or recombinant DIRS2 polypeptide comprising at least three distinct nonoverlapping segments of at least ten amino acids identical to segments of SEQ ID NO: 4;
- 20 f) a substantially pure or recombinant DIRS2 polypeptide comprising at least two distinct nonoverlapping segments of at least eleven amino acids identical to segments of SEQ ID NO: 4;
- 25 g) a natural sequence DIRS2 comprising SEQ ID NO: 4; or
- h) a fusion polypeptide comprising DIRS2 sequence.
2. The substantially pure or isolated antigenic:
- 30 A) DIRS1 polypeptide of Claim 1, wherein said distinct nonoverlapping segments of identity:
- a) include one of at least eight amino acids;
- b) include one of at least four amino acids and a second of at least five amino acids;
- 35 c) include at least three segments of at least four, five, and six amino acids, or
- d) include one of at least twelve amino acids; or

B) DIRS2 polypeptide of Claim 1, wherein said distinct nonoverlapping segments of identity:

- a) include one of at least thirteen amino acids;
- b) include one of at least eleven amino acids and a
5 second of at least thirteen amino acids;
- c) include at least three segments of at least ten, eleven, and twelve amino acids; or
- d) include one of at least twenty-five amino acids.

10 3. The composition of matter of Claim 1, wherein said:

- a) DIRS1 polypeptide:
 - i) comprises a mature sequence of Table 1;
 - ii) is an unglycosylated form of DIRS1;
 - 15 iii) is from a primate, such as a human;
 - iv) comprises at least seventeen amino acids of SEQ ID NO: 2;
 - v) exhibits at least four nonoverlapping segments of at least seven amino acids of
20 SEQ ID NO: 2;
 - vi) is a natural allelic variant of DIRS1;
 - vii) has a length at least about 30 amino acids;
 - viii) exhibits at least two non-overlapping epitopes which are specific for a primate
25 DIRS1;
 - ix) is glycosylated;
 - x) has a molecular weight of at least 30 kD with natural glycosylation;
 - xi) is a synthetic polypeptide;
 - 30 xii) is attached to a solid substrate;
 - xiii) is conjugated to another chemical moiety;
 - xiv) is a 5-fold or less substitution from natural sequence; or
 - xv) is a deletion or insertion variant from a
35 natural sequence; or
- b) DIRS2 polypeptide:
 - i) comprises a mature sequence of Table 2;

- ii) is an unglycosylated form of DIRS2;
- iii) is from a primate, such as a human;
- iv) comprises at thirty-five amino acids of SEQ ID NO: 4;
- 5 v) exhibits at least four nonoverlapping segments of at least twelve amino acids of SEQ ID NO: 4;
- vi) is a natural allelic variant of DIRS2;
- vii) has a length at least about 30 amino acids;
- 10 viii) exhibits at least two non-overlapping epitopes which are specific for a primate DIRS2;
- ix) is glycosylated;
- x) has a molecular weight of at least 30 kD with
- 15 natural glycosylation;
- xi) is a synthetic polypeptide;
- xii) is attached to a solid substrate;
- xiii) is conjugated to another chemical moiety;
- xiv) is a 5-fold or less substitution from
- 20 natural sequence; or
- xv) is a deletion or insertion variant from a natural sequence.

4. A composition comprising:
- 25 a) a substantially pure DIRS1 and another Interferon Receptor family member;
 - b) a substantially pure DIRS2 and another Interferon Receptor family member;
 - c) a sterile DIRS1 polypeptide of Claim 1;
 - 30 d) a sterile DIRS2 polypeptide of Claim 1;
 - e) said DIRS1 polypeptide of Claim 1 and a carrier, wherein said carrier is:
 - i) an aqueous compound, including water, saline, and/or buffer; and/or
 - 35 ii) formulated for oral, rectal, nasal, topical, or parenteral administration; or

- f) said DIRS2 polypeptide of Claim 1 and a carrier,
wherein said carrier is:
- 5 i) an aqueous compound, including water, saline,
 and/or buffer; and/or
- ii) formulated for oral, rectal, nasal, topical,
 or parenteral administration.

5. The fusion polypeptide of Claim 1, comprising:
- 10 a) mature protein sequence of Table 1;
- b) mature protein sequence of Table 2;
- c) a detection or purification tag, including a FLAG, His6, or Ig sequence; or
- d) sequence of another interferon receptor protein.

- 15 6. A kit comprising a polypeptide of Claim 1, and:
- a) a compartment comprising said protein or
 polypeptide; or
- b) instructions for use or disposal of reagents in
 said kit.

7. A binding compound comprising an antigen binding site from an antibody, which specifically binds to a natural:

- A) DIRS1 polypeptide of Claim 1, wherein:
- 25 a) said binding compound is in a container;
- b) said DIRS1 polypeptide is from a human;
- c) said binding compound is an Fv, Fab, or Fab2
 fragment;
- d) said binding compound is conjugated to another
30 chemical moiety; or
- e) said antibody:
- i) is raised against a peptide sequence of a
 mature polypeptide of Table 1;
- ii) is raised against a mature DIRS1;
- 35 iii) is raised to a purified human DIRS1;
- iv) is immunoselected;
- v) is a polyclonal antibody;

- vi) binds to a denatured DIRS1;
vii) exhibits a K_d to antigen of at least 30 μM ;
viii) is attached to a solid substrate,
including a bead or plastic membrane;
ix) is in a sterile composition; or
x) is detectably labeled, including a
radioactive or fluorescent label; or
- 5
- B) DIRS2 polypeptide of Claim 1, wherein:
- a) said binding compound is in a container;
b) said DIRS2 protein is from a human;
c) said binding compound is an Fv, Fab, or Fab2
fragment;
d) said binding compound is conjugated to another
chemical moiety; or
e) said antibody:
i) is raised against a peptide sequence of a
mature polypeptide of Table 2;
ii) is raised against a mature DIRS2;
iii) is raised to a purified human DIRS2;
iv) is immunoselected;
v) is a polyclonal antibody;
vi) binds to a denatured DIRS2;
vii) exhibits a K_d to antigen of at least 30 μM ;
viii) is attached to a solid substrate,
including a bead or plastic membrane;
ix) is in a sterile composition; or
x) is detectably labeled, including a
radioactive or fluorescent label.
- 10
- 15
- 20
- 25
- 30
8. A kit comprising said binding compound of Claim
7, and:
a) a compartment comprising said binding compound; or
b) instructions for use or disposal of reagents in
said kit.
- 35

9. A method of producing an antigen:antibody complex, comprising contacting under appropriate conditions:

- 5 a) a primate DIRS1 polypeptide with an antibody of Claim 7A; or
- b) a primate DIRS2 polypeptide with an antibody of Claim 7B;

thereby allowing said complex to form.

10 10. The method of Claim 9, wherein:

- a) said complex is purified from other interferon receptors;
- b) said complex is purified from other antibody;
- 15 c) said contacting is with a sample comprising an interferon;
- d) said contacting allows quantitative detection of said antigen;
- e) said contacting is with a sample comprising said antibody; or
- 20 f) said contacting allows quantitative detection of said antibody.

11. A composition comprising:

- a) a sterile binding compound of Claim 7; or
- 25 b) said binding compound of Claim 7 and a carrier, wherein said carrier is:
 - i) an aqueous compound, including water, saline, and/or buffer; and/or
 - ii) formulated for oral, rectal, nasal, topical,
 - 30 or parenteral administration.

12. An isolated or recombinant nucleic acid encoding said:

- A) DIRS1 polypeptide of Claim 1, wherein said:
 - 35 a) DIRS1 is from a human; or
 - b) said nucleic acid:

- 5 i) encodes an antigenic peptide sequence of
 Table 1;
 ii) encodes a plurality of antigenic peptide
 sequences of Table 1;
 iii) exhibits identity over at least thirteen
 nucleotides to a natural cDNA encoding said
 segment;
 iv) is an expression vector;
 v) further comprises an origin of replication;
10 vi) is from a natural source;
 vii) comprises a detectable label;
 viii) comprises synthetic nucleotide sequence;
 ix) is less than 6 kb, preferably less than 3
 kb;
15 x) is from a primate;
 xi) comprises a natural full length coding
 sequence;
 xii) is a hybridization probe for a gene
 encoding said DIRS1; or
20 xiii) is a PCR primer, PCR product, or
 mutagenesis primer; or

B) DIRS2 polypeptide of Claim 1, wherein said:

- a) DIRS2 is from a human; or
 b) said nucleic acid:
25 i) encodes an antigenic peptide sequence of
 Table 2;
 ii) encodes a plurality of antigenic peptide
 sequences of Table 2;
 iii) exhibits identity over at least 30
30 nucleotides to a natural cDNA encoding said
 segment;
 iv) is an expression vector;
 v) further comprises an origin of replication;
 vi) is from a natural source;
35 vii) comprises a detectable label;
 viii) comprises synthetic nucleotide sequence;

- ix) is less than 6 kb, preferably less than 3 kb;
- x) is from a primate;
- xi) comprises a natural full length coding sequence;
- xii) is a hybridization probe for a gene encoding said DIRS2; or
- xiii) is a PCR primer, PCR product, or mutagenesis primer.
13. A cell or tissue comprising said recombinant nucleic acid of Claim 12.
14. The cell of Claim 13, wherein said cell is:
- a) a prokaryotic cell;
 - b) a eukaryotic cell;
 - c) a bacterial cell;
 - d) a yeast cell;
 - e) an insect cell;
 - f) a mammalian cell;
 - g) a mouse cell;
 - h) a primate cell; or
 - i) a human cell.
15. A kit comprising said nucleic acid of Claim 12, and:
- a) a compartment comprising said nucleic acid;
 - b) a compartment further comprising a primate DIRS1 polypeptide;
 - c) a compartment further comprising a primate DIRS2 polypeptide; or
 - d) instructions for use or disposal of reagents in said kit.
16. A nucleic acid which:

- 5 a) hybridizes under wash conditions of 30 minutes at
30° C and less than 2M salt to the coding portion
of SEQ ID NO: 1;
- b) hybridizes under wash conditions of 30 minutes at
30° C and less than 2M salt to the coding portion
of SEQ ID NO: 3;
- 10 c) exhibits identity over a stretch of at least about
30 nucleotides to a primate DIRS1; or
- d) exhibits identity over a stretch of at least about
30 nucleotides to a primate DIRS2.
17. The nucleic acid of Claim 16, wherein:
- 15 a) said wash conditions are at 45° C and/or 500 mM
salt; or
- b) said stretch is at least 55 nucleotides.
18. The nucleic acid of Claim 16, wherein:
- 20 a) said wash conditions are at 55° C and/or 150 mM
salt; or
- b) said stretch is at least 75 nucleotides.
19. A method of modulating physiology or development
of a cell or tissue culture cells comprising contacting
said cell with an agonist or antagonist of a mammalian
25 DIRS1 or DIRS2.
20. The method of Claim 19, wherein said cell is
transformed with a nucleic acid encoding a DIRS1 or DIRS2
and another cytokine receptor subunit.

SEQUENCE SUBMISSION

5 SEQ ID NO: 1 is primate DIRS1 nucleotide sequence.
SEQ ID NO: 2 is primate DIRS1 polypeptide sequence.
SEQ ID NO: 3 is primate DIRS2 nucleotide sequence.
SEQ ID NO: 4 is primate DIRS2 polypeptide sequence.
SEQ ID NO: 5 is primate IFN γ receptor subunit beta polypeptide sequence.
10 SEQ ID NO: 6 is primate CRF2-4 receptor subunit polypeptide sequence.

(1) GENERAL INFORMATION:

15 (i) APPLICANT: Parham, Christi L.
Moore, Kevin W.
Murgolo, Nicholas J.
Bazan, J. Fernando

20 (ii) TITLE OF INVENTION: Mammalian Receptor Proteins; Related
Reagents and Methods

(iii) NUMBER OF SEQUENCES: 6

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30 (F) ZIP: 94304-1104

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
35 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
40 (B) FILING DATE: 08-MAR-1999
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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45 (B) REGISTRATION NUMBER: 34,090
(C) REFERENCE/DOCKET NUMBER: DX0804K

(ix) TELECOMMUNICATION INFORMATION:
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50 (B) TELEFAX: (650)496-1200

(2) INFORMATION FOR SEQ ID NO:1:

55 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1381 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

10 (A) NAME/KEY: CDS
(B) LOCATION: 132..1064

(ix) FEATURE:

15 (A) NAME/KEY: misc_feature
(B) LOCATION: 567
(D) OTHER INFORMATION: /note= "nucleotides 567, 573, 1336, 1342, and 1369 designated C, but each may be A, C, G, or T"

(ix) FEATURE:

20 (A) NAME/KEY: misc_feature
(B) LOCATION: 643
(D) OTHER INFORMATION: /note= "nucleotides 643, 1287, and 1290 designated C, but each may be C or G"

(ix) FEATURE:

25 (A) NAME/KEY: misc_feature
(B) LOCATION: 772
(D) OTHER INFORMATION: /note= "nucleotides 772, 806, and 1261 designated G, but each may be A or G"

(ix) FEATURE:

30 (A) NAME/KEY: misc_feature
(B) LOCATION: 1236
(D) OTHER INFORMATION: /note= "nucleotides 1236, 1260, 1282, and 1289 are designated T, but each may be G or T"

(ix) FEATURE:

35 (A) NAME/KEY: misc_feature
(B) LOCATION: 1247
(D) OTHER INFORMATION: /note= "nucleotides 1247, 1257, 1293, and 1302 designated C, but each may be C or T"

(ix) FEATURE:

40 (A) NAME/KEY: misc_feature
(B) LOCATION: 1266
(D) OTHER INFORMATION: /note= "nucleotides 1266 and 1298 designated T, but each may be A or T"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

50 TCGACCCACG CGTCCGCGCT GCGACTCAGA CCTCAGCTCC AACATATGCA TTCTGAAGAA 60
AGATGGCTGA GATGGACAGA ATGCTTTATT TTGGAAAGAA ACAATGTTCT AGGTCAAAC 120
55 GAGTCTACCA A ATG CAG ACT TTC ACA ATG GTT CTA GAA GAA ATC TGG ACA 170

	GAA	TGT	GTG	GAG	GTG	CAA	GGA	GAG	GCC	ATT	CCC	CTG	GTA	CTG	GCC	CTG	842
	Glu	Cys	Val	Glu	Val	Gln	Gly	Glu	Ala	Ile	Pro	Leu	Val	Leu	Ala	Leu	
				225					230					235			
5	TTT	GCC	TTT	GTT	GGC	TTC	ATG	CTG	ATC	CTT	GTG	GTC	GTG	CCA	CTG	TTC	890
	Phe	Ala	Phe	Val	Gly	Phe	Met	Leu	Ile	Leu	Val	Val	Val	Pro	Leu	Phe	
			240					245					250				
10	GTC	TGG	AAA	ATG	GGC	CGG	CTG	CTC	CAG	TAC	TCC	TGT	TGC	CCC	GTG	GTG	938
	Val	Trp	Lys	Met	Gly	Arg	Leu	Leu	Gln	Tyr	Ser	Cys	Cys	Pro	Val	Val	
		255					260					265					
15	GTC	CTC	CCA	GAC	ACC	TTG	AAA	ATA	ACC	AAT	TCA	CCC	CAG	AAG	TTA	ATC	986
	Val	Leu	Pro	Asp	Thr	Leu	Lys	Ile	Thr	Asn	Ser	Pro	Gln	Lys	Leu	Ile	
	270					275					280					285	
20	AGC	TGC	AGA	AGG	GAG	GAG	GTG	GAT	GCC	TGT	GCC	ACG	GCT	GTG	ATG	TCT	1034
	Ser	Cys	Arg	Arg	Glu	Glu	Val	Asp	Ala	Cys	Ala	Thr	Ala	Val	Met	Ser	
				290						295					300		
	CCT	GAG	GAA	CTC	CTC	AGG	GCC	TGG	ATC	TCA	TAGGTTT	TGCG	GAAGGG	CCCA			1084
	Pro	Glu	Glu	Leu	Leu	Arg	Ala	Trp	Ile	Ser							
			305						310								
25	GGTGAAGCCG	AGAACCTGGT	CTGCATGACA	TGGAAACCAT	GAGGGGACAA	GTTGTGTTTC											1144
	TGTTTTCCGC	CACGGACAAG	GGATGAGAGA	AGTAGGAAGA	GCCTGTTGTC	TACAAGTCTA											1204
	GAAGCAACCA	TCAGAGGCAG	GGTGGTTTGT	CTAACAGAAC	AACTGACTGA	GGCTATGGGG											1264
30	GTTGTGACCT	CTAGACTTTG	GGCTTCCACT	TGCTTGCTG	AGCAACCCTG	GGAAAAGTGA											1324
	CTTCATCCCT	TCGGTCCCAA	GTTTTCTCAT	CTGTAATGGG	GGATCCCTAC	AAAAGTGA											1381
35																	
	(2) INFORMATION FOR SEQ ID NO:2:																
	(i) SEQUENCE CHARACTERISTICS:																
	(A) LENGTH: 311 amino acids																
40	(B) TYPE: amino acid																
	(D) TOPOLOGY: linear																
	(ii) MOLECULE TYPE: protein																
45	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:																
	Met	Gln	Thr	Phe	Thr	Met	Val	Leu	Glu	Glu	Ile	Trp	Thr	Ser	Leu	Phe	
	1				5					10					15		
50	Met	Trp	Phe	Phe	Tyr	Ala	Leu	Ile	Pro	Cys	Leu	Leu	Thr	Asp	Glu	Val	
			20														

Lys His Leu Leu Met Trp Ser Pro Val Ile Ala Pro Gly Glu Thr Val
 50 55 60
 Tyr Tyr Ser Val Glu Tyr Gln Gly Glu Tyr Glu Ser Leu Tyr Thr Ser
 5 65 70 75 80
 His Ile Trp Ile Pro Ser Ser Trp Cys Ser Leu Thr Glu Gly Pro Glu
 85 90 95
 10 Cys Asp Val Thr Asp Asp Ile Thr Ala Thr Val Pro Tyr Asn Leu Arg
 100 105 110
 Val Arg Ala Thr Leu Gly Ser Gln Thr Ser Ala Trp Ser Ile Leu Lys
 115 120 125
 15 His Pro Phe Asn Arg Asn Ser Thr Ile Leu Thr Arg Pro Gly Met Glu
 130 135 140
 Ile Pro Lys His Gly Phe His Leu Val Ile Glu Leu Glu Asp Leu Gly
 20 145 150 155 160
 Pro Gln Phe Glu Phe Leu Val Ala Tyr Trp Thr Arg Glu Pro Gly Ala
 165 170 175
 25 Glu Glu His Val Lys Met Val Arg Ser Gly Gly Ile Pro Val His Leu
 180 185 190
 Glu Thr Met Glu Pro Gly Ala Ala Tyr Cys Val Lys Ala Gln Thr Phe
 195 200 205
 30 Val Lys Ala Ile Gly Arg Tyr Ser Ala Phe Ser Gln Thr Glu Cys Val
 210 215 220
 Glu Val Gln Gly Glu Ala Ile Pro Leu Val Leu Ala Leu Phe Ala Phe
 35 225 230 235 240
 Val Gly Phe Met Leu Ile Leu Val Val Val Pro Leu Phe Val Trp Lys
 245 250 255
 40 Met Gly Arg Leu Leu Gln Tyr Ser Cys Cys Pro Val Val Val Leu Pro
 260 265 270
 Asp Thr Leu Lys Ile Thr Asn Ser Pro Gln Lys Leu Ile Ser Cys Arg
 275 280 285
 45 Arg Glu Glu Val Asp Ala Cys Ala Thr Ala Val Met Ser Pro Glu Glu
 290 295 300
 Leu Leu Arg Ala Trp Ile Ser
 50 305 310

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

55 (A) LENGTH: 1244 base pairs

663020"0459200

(B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

10 (A) NAME/KEY: CDS
 (B) LOCATION: 2..694

(ix) FEATURE:

15 (A) NAME/KEY: misc_feature
 (B) LOCATION: 193
 (D) OTHER INFORMATION: /note= "nucleotide 193 designated
 C, may be C or T"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

20 C CGG GTC GAC CCA CGC GTC CGC CTG GTT TCC CCC TGG CTG ACA GTG 46
 Arg Val Asp Pro Arg Val Arg Leu Val Ser Pro Trp Leu Thr Val
 1 5 10 15

25 CCT TGG TTC CTG TCC TGT TGG AAT GTT ACC ATT GGG CCT CCT GAG AGC 94
 Pro Trp Phe Leu Ser Cys Trp Asn Val Thr Ile Gly Pro Pro Glu Ser
 20 25 30

30 ATC TGG GTG ACG CCG GGA GAA GCC TCC CTC ATC ATC AGG TTC TCC TCT 142
 Ile Trp Val Thr Pro Gly Glu Ala Ser Leu Ile Ile Arg Phe Ser Ser
 35 40 45

35 CCC TTC GAC GTC CCT CCC AAC CTG GGC TAT TTC CAG TAC TAT GTC CAT 190
 Pro Phe Asp Val Pro Pro Asn Leu Gly Tyr Phe Gln Tyr Tyr Val His
 50 55 60

TAC TGG GAA AAG GCG GGA ATC CAA AAG GTT AAA GGT CCT TTC AAG AGC 238
 Tyr Trp Glu Lys Ala Gly Ile Gln Lys Val Lys Gly Pro Phe Lys Ser
 65 70 75

40 AAC TCC ATC GTG TTG GAT GGC TTG AGA CCC TTA AGA GAA TAC TGT TTA 286
 Asn Ser Ile Val Leu Asp Gly Leu Arg Pro Leu Arg Glu Tyr Cys Leu
 80 85 90 95

45 CAA GTG AAG GCG CAT CTC TTT CGC ACA TCC TGC AAC ACC TCT AGG CCC 334
 Gln Val Lys Ala His Leu Phe Arg Thr Ser Cys Asn Thr Ser Arg Pro
 100 105 110

50 GGC CGC TTA AGC AAC ATA ACT TGC TAC GAA ACA ATG ATG GAT GCC ACT 382
 Gly Arg Leu Ser Asn Ile Thr Cys Tyr Glu Thr Met Met Asp Ala Thr
 115 120 125

55 ACG AAG CTT CAA CAA GTC ATC CTC ATC GCC GTG GGA GTC TTT CTG TCG 430
 Thr Lys Leu Gln Gln Val Ile Leu Ile Ala Val Gly Val Phe Leu Ser
 130 135 140

CTG GCG GCG CTG GCG GGG GGC TGT TTC TTC CTG GTG CTG AGA TAC AAA 478
Leu Ala Ala Leu Ala Gly Gly Cys Phe Phe Leu Val Leu Arg Tyr Lys
145 150 155

5 GGC CTG GTG AAA TAC TGG TTT CAC TCT CCG CCA AGC ATC CCA TCA CAA 526
Gly Leu Val Lys Tyr Trp Phe His Ser Pro Pro Ser Ile Pro Ser Gln
160 165 170 175

10 ATC GAA GAG TAT CTG AAG GAC CCG AGC CAG CCT ATC CTA GAG GCC CTG 574
Ile Glu Glu Tyr Leu Lys Asp Pro Ser Gln Pro Ile Leu Glu Ala Leu
180 185 190

15 GAC AAG GAC ACG TCA CCA ACA GAT GAT GCC TGG GAC TTG GTG TCT GTT 622
Asp Lys Asp Thr Ser Pro Thr Asp Asp Ala Trp Asp Leu Val Ser Val
195 200 205

20 GTT GCA TTT CCA GCA AAG GAG CAA GAA GAT GTT CCC CAA AGC ACT TTG 670
Val Ala Phe Pro Ala Lys Glu Gln Glu Asp Val Pro Gln Ser Thr Leu
210 215 220

ACC CAA AAC TCT GGT GCG GTC TGC TAGCCTGTGG GGTAAAGGGCT CTGAGCCGAG 724
Thr Gln Asn Ser Gly Ala Val Cys
225 230

25 GAAGCTGCTG ATGTCCATGT CAGCACTTTA TGGAATCCGG TCCTCCATTT TCCTGTCCCC 784

AAAAGGCCCG TCAGTGCCTG TGAAGATGTA ACGGGTCTCA TGGGGGCGAC AAGCTTATTG 844

30 ATTTTTTTCT TCAAACCTAAG AGTTTTCTAA TCATACGCGT TTTTAGAATA ATTCTACAGA 904

TATGTCCCCG AAAGATTAAG ATTTCTCTTA AACACTAAAA AGACATGTAA TTATTTGTTA 964

35 GCAAATGGGC GTCTGGCAGC CCTCTGACAC TTTTTCGTCA GCAGCCAGGA CACGAGGTCC 1024

CCTCCTTGAT GAAGCCCCTC GGGCAGACCA TGTCACCTGT CCCAGCCTGC CCCAAGAAGG 1084

GACATTAAGT GGCCCTTCTT CATATCCAAA CACCTGGCTT GAAATGTGAT TAGCCCTGTA 1144

40 AATAGTTTCA CAGAGATTAA GCCTTTTTTTT CCCCCAAGTT AGGAATAAAA GACTATAATT 1204

AACTTTTTTAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 1244

45 (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 231 amino acids

(B) TYPE: amino acid

50 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

55

45

(i) SEQUENCE CHARACTERISTICS:

50

55

[illegible]

Variable	Mean	SD	Min	Max	Median	Q1	Q3	Mode	Skewness	Kurtosis	Jarque-Bera	Prob > Chi-Sq
Age	34.5	10.5	20	65	35	30	40	35	-0.1	3.2	0.98	0.60
Gender	1.5	0.5	1	2	1.5	1.5	1.5	1.5	0.0	0.0	0.95	0.62
Marital Status	2.5	1.0	1	4	2.5	2.0	3.0	2.5	-0.2	1.5	0.92	0.65
Education	12.5	2.0	9	16	12.5	11.5	13.5	12.5	-0.1	2.5	0.97	0.61
Income	1500	500	500	3000	1200	800	1800	1200	-0.3	4.0	0.94	0.63
Health	3.5	1.0	1	5	3.5	3.0	4.0	3.5	-0.1	1.0	0.99	0.58
Stress	4.0	1.5	1	6	4.0	3.0	5.0	4.0	-0.2	2.0	0.96	0.64
Life Satisfaction	3.0	1.0	1	5	3.0	2.5	3.5	3.0	-0.1	1.5	0.98	0.59
Work Satisfaction	3.5	1.0	1	5	3.5	3.0	4.0	3.5	-0.1	1.0	0.99	0.58
Family Satisfaction	3.0	1.0	1	5	3.0	2.5	3.5	3.0	-0.1	1.5	0.98	0.59
Community Satisfaction	3.0	1.0	1	5	3.0	2.5	3.5	3.0	-0.1	1.5	0.98	0.59
Overall Satisfaction	3.0	1.0	1	5	3.0	2.5	3.5	3.0	-0.1	1.5	0.98	0.59

HUMAN RECEPTOR PROTEINS; RELATED REAGENTS AND METHODS

5

ABSTRACT

10 Nucleic acids encoding mammalian, e.g., primate or rodent receptors, purified receptor proteins and fragments thereof. Antibodies, both polyclonal and monoclonal, are also provided. Methods of using the compositions for both diagnostic and therapeutic utilities are provided.

[illegible]

(ii) TITLE OF INVENTION: Mammalian Receptor Proteins; Related Reagents and Methods

(iv) CORRESPONDENCE ADDRESS:

- (v) COMPUTER READABLE FORM:

- (vi) CURRENT APPLICATION DATA:

- (viii) ATTORNEY/AGENT INFORMATION:

- (ix) TELECOMMUNICATION INFORMATION:

- (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1381 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

[illegible]

(A) NAME/KEY: misc_feature
(B) LOCATION: 567
(D) OTHER INFORMATION: /note= "nucleotides 567, 573, 1336, 1342, and 1369 designated C, but each may be A, C, G, or T"

(A) NAME/KEY: misc_feature
(B) LOCATION: 643
(D) OTHER INFORMATION: /note= "nucleotides 643, 1287, and 1290 designated C, but each may be C or G"

(A) NAME/KEY: misc_feature
(B) LOCATION: 772
(D) OTHER INFORMATION: /note= "nucleotides 772, 806, and 1261 designated G, but each may be A or G"

(A) NAME/KEY: misc_feature
(B) LOCATION: 1236
(D) OTHER INFORMATION: /note= "nucleotides 1236, 1260, 1282, and 1289 are designated T, but each may be G or T"

(A) NAME/KEY: misc_feature
(B) LOCATION: 1247
(D) OTHER INFORMATION: /note= "nucleotides 1247, 1257, 1293, and 1302 designated C, but each may be C or T"

(A) NAME/KEY: misc_feature
(B) LOCATION: 1266
(D) OTHER INFORMATION: /note= "nucleotides 1266 and 1298 designated T, but each may be A or T"

TCGACCCACG	CGTCCGCGCT	GCGACTCAGA	CCTCAGCTCC	AACATATGCA	TTCTGAAGAA	60										
AGATGGCTGA	GATGGACAGA	ATGCTTTATT	TTGGAAAGAA	ACAATGTTCT	AGGTCAAAC	120										
GAGTCTACCA	A	ATG	CAG	ACT	TTC	ACA	ATG	GTT	CTA	GAA	GAA	ATC	TGG	ACA	170	
		Met	Gln	Thr	Phe	Thr	Met	Val	Leu	Glu	Glu	Ile	Trp	Thr		
		1				5						10				
AGT	CTT	TTC	ATG	TGG	TTT	TTC	TAC	GCA	TTG	ATT	CCA	TGT	TTG	CTC	ACA	218
Ser	Leu	Phe	Met	Trp	Phe	Phe	Tyr	Ala	Leu	Ile	Pro	Cys	Leu	Leu	Thr	
		15					20					25				

GAT Asp 30	GAA Glu	GTG Val	GCC Ala	ATT Ile	CTG Leu	CCT Pro	GCC Ala	CCT Pro	CAG Gln	AAC Asn	CTC Leu	TCT Ser	GTA Val	CTC Leu	TCA Ser	266
ACC Thr	AAC Asn	ATG Met	AAG Lys	CAT His	CTC Leu	TTG Leu	ATG Met	TGG Trp	AGC Ser	CCA Pro	GTG Val	ATC Ile	GCG Ala	CCT Pro	GGA Gly	314
GAA Glu	ACA Thr	GTG Val	TAC Tyr	TAT Tyr	TCT Ser	GTC Val	GAA Glu	TAC Tyr	CAG Gln	GGG Gly	GAG Glu	TAC Tyr	GAG Glu	AGC Ser	CTG Leu	362
TAC Tyr	ACG Thr	AGC Ser	CAC His	ATC Ile	TGG Trp	ATC Ile	CCC Pro	AGC Ser	AGC Ser	TGG Trp	TGC Cys	TCA Ser	CTC Leu	ACT Thr	GAA Glu	410
GGT Gly	CCT Pro	GAG Glu	TGT Cys	GAT Asp	GTC Val	ACT Thr	GAT Asp	GAC Asp	ATC Ile	ACG Thr	GCC Ala	ACT Thr	GTG Val	CCA Pro	TAC Tyr	458
AAC Asn 110	CTT Leu	CGT Arg	GTC Val	AGG Arg	GCC Ala	ACA Thr	TTG Leu	GGC Gly	TCA Ser	CAG Gln	ACC Thr	TCA Ser	GCC Ala	TGG Trp	AGC Ser	506
ATC Ile	CTG Leu	AAG Lys	CAT His	CCC Pro	TTT Phe	AAT Asn	AGA Arg	AAC Asn	TCA Ser	ACC Thr	ATC Ile	CTT Leu	ACC Thr	CGA Arg	CCT Pro	554
GGG Gly	ATG Met	GAG Glu	ATC Ile	CCC Pro	AAA Lys	CAT His	GGC Gly	TTC Phe	CAC His	CTG Leu	GTT Val	ATT Ile	GAG Glu	CTG Leu	GAG Glu	602
GAC Asp	CTG Leu	GGG Gly	CCC Pro	CAG Gln	TTT Phe	GAG Glu	TTC Phe	CTT Leu	GTG Val	GCC Ala	TAC Tyr	TGG Trp	ACG Thr	AGG Arg	GAG Glu	650
CCT Pro	GGT Gly	GCC Ala	GAG Glu	GAA Glu	CAT His	GTC Val	AAA Lys	ATG Met	GTG Val	AGG Arg	AGT Ser	GGG Gly	GGT Gly	ATT Ile	CCA Pro	698
GTG Val 190	CAC His	CTA Leu	GAA Glu	ACC Thr	ATG Met	GAG Glu	CCA Pro	GGG Gly	GCT Ala	GCA Ala	TAC Tyr	TGT Cys	GTG Val	AAG Lys	GCC Ala	746
CAG Gln	ACA Thr	TTC Phe	GTG Val	AAG Lys	GCC Ala	ATT Ile	GGG Gly	AGG Arg	TAC Tyr	AGC Ser	GCC Ala	TTC Phe	AGC Ser	CAG Gln	ACA Thr	794
GAA Glu	TGT Cys	GTG Val	GAG Glu	GTG Val	CAA Gln	GGA Gly	GAG Glu	GCC Ala	ATT Ile	CCC Pro	CTG Leu	GTA Val	CTG Leu	GCC Ala	CTG Leu	842
TTT Phe	GCC Ala	TTT Phe	GTT Val	GGC Gly	TTC Phe	ATG Met	CTG Leu	ATC Ile	CTT Leu	GTG Val	GTC Val	GTG Val	CCA Pro	CTG Leu	TTC Phe	890

240	245	250	
GTC TGG AAA ATG GGC CGG CTG CTC CAG TAC TCC TGT TGC CCC GTG GTG			938
Val Trp Lys Met Gly Arg Leu Leu Gln Tyr Ser Cys Cys Pro Val Val			
255	260	265	
GTC CTC CCA GAC ACC TTG AAA ATA ACC AAT TCA CCC CAG AAG TTA ATC			986
Val Leu Pro Asp Thr Leu Lys Ile Thr Asn Ser Pro Gln Lys Leu Ile			
270	275	280	285
AGC TGC AGA AGG GAG GAG GTG GAT GCC TGT GCC ACG GCT GTG ATG TCT			1034
Ser Cys Arg Arg Glu Glu Val Asp Ala Cys Ala Thr Ala Val Met Ser			
290	295	300	
CCT GAG GAA CTC CTC AGG GCC TGG ATC TCA TAGGTTTGCG GAAGGGCCCA			1084
Pro Glu Glu Leu Leu Arg Ala Trp Ile Ser			
305	310		
GGTGAAGCCG AGAACCTGGT CTGCATGACA TGGAAACCAT GAGGGGACAA GTTGTGTTTC			1144
TGTTTTCCGC CACGGACAAG GGATGAGAGA AGTAGGAAGA GCCTGTTGTC TACAAGTCTA			1204
GAAGCAACCA TCAGAGGCAG GGTGGTTTGT CTAACAGAAC AACTGACTGA GGCTATGGGG			1264
GTGTGACCT CTAGACTTTG GGCTTCCACT TGCTTGGCTG AGCAACCCTG GGAAAAGTGA			1324
CTTCATCCCT TCGGTCCCAA GTTTTCTCAT CTGTAATGGG GGATCCCTAC AAAACTG			1381

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 311 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Gln Thr Phe Thr Met Val Leu Glu Glu Ile Trp Thr Ser Leu Phe	
1 5 10 15	
Met Trp Phe Phe Tyr Ala Leu Ile Pro Cys Leu Leu Thr Asp Glu Val	
20 25 30	
Ala Ile Leu Pro Ala Pro Gln Asn Leu Ser Val Leu Ser Thr Asn Met	
35 40 45	
Lys His Leu Leu Met Trp Ser Pro Val Ile Ala Pro Gly Glu Thr Val	
50 55 60	
Tyr Tyr Ser Val Glu Tyr Gln Gly Glu Tyr Glu Ser Leu Tyr Thr Ser	
65 70 75 80	

His Ile Trp Ile Pro Ser Ser Trp Cys Ser Leu Thr Glu Gly Pro Glu
 85 90 95
 Cys Asp Val Thr Asp Asp Ile Thr Ala Thr Val Pro Tyr Asn Leu Arg
 100 105 110
 Val Arg Ala Thr Leu Gly Ser Gln Thr Ser Ala Trp Ser Ile Leu Lys
 115 120 125
 His Pro Phe Asn Arg Asn Ser Thr Ile Leu Thr Arg Pro Gly Met Glu
 130 135 140
 Ile Pro Lys His Gly Phe His Leu Val Ile Glu Leu Glu Asp Leu Gly
 145 150 155 160
 Pro Gln Phe Glu Phe Leu Val Ala Tyr Trp Thr Arg Glu Pro Gly Ala
 165 170 175
 Glu Glu His Val Lys Met Val Arg Ser Gly Gly Ile Pro Val His Leu
 180 185 190
 Glu Thr Met Glu Pro Gly Ala Ala Tyr Cys Val Lys Ala Gln Thr Phe
 195 200 205
 Val Lys Ala Ile Gly Arg Tyr Ser Ala Phe Ser Gln Thr Glu Cys Val
 210 215 220
 Glu Val Gln Gly Glu Ala Ile Pro Leu Val Leu Ala Leu Phe Ala Phe
 225 230 235 240
 Val Gly Phe Met Leu Ile Leu Val Val Val Pro Leu Phe Val Trp Lys
 245 250 255
 Met Gly Arg Leu Leu Gln Tyr Ser Cys Cys Pro Val Val Val Leu Pro
 260 265 270
 Asp Thr Leu Lys Ile Thr Asn Ser Pro Gln Lys Leu Ile Ser Cys Arg
 275 280 285
 Arg Glu Glu Val Asp Ala Cys Ala Thr Ala Val Met Ser Pro Glu Glu
 290 295 300
 Leu Leu Arg Ala Trp Ile Ser
 305 310

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1244 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 2..694

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 193

(D) OTHER INFORMATION: /note= "nucleotide 193 designated
C, may be C or T"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

C CGG GTC GAC CCA CGC GTC CGC CTG GTT TCC CCC TGG CTG ACA GTG	46
Arg Val Asp Pro Arg Val Arg Leu Val Ser Pro Trp Leu Thr Val	
1 5 10 15	
CCT TGG TTC CTG TCC TGT TGG AAT GTT ACC ATT GGG CCT CCT GAG AGC	94
Pro Trp Phe Leu Ser Cys Trp Asn Val Thr Ile Gly Pro Pro Glu Ser	
20 25 30	
ATC TGG GTG ACG CCG GGA GAA GCC TCC CTC ATC ATC AGG TTC TCC TCT	142
Ile Trp Val Thr Pro Gly Glu Ala Ser Leu Ile Ile Arg Phe Ser Ser	
35 40 45	
CCC TTC GAC GTC CCT CCC AAC CTG GGC TAT TTC CAG TAC TAT GTC CAT	190
Pro Phe Asp Val Pro Pro Asn Leu Gly Tyr Phe Gln Tyr Tyr Val His	
50 55 60	
TAC TGG GAA AAG GCG GGA ATC CAA AAG GTT AAA GGT CCT TTC AAG AGC	238
Tyr Trp Glu Lys Ala Gly Ile Gln Lys Val Lys Gly Pro Phe Lys Ser	
65 70 75	
AAC TCC ATC GTG TTG GAT GGC TTG AGA CCC TTA AGA GAA TAC TGT TTA	286
Asn Ser Ile Val Leu Asp Gly Leu Arg Pro Leu Arg Glu Tyr Cys Leu	
80 85 90 95	
CAA GTG AAG GCG CAT CTC TTT CGC ACA TCC TGC AAC ACC TCT AGG CCC	334
Gln Val Lys Ala His Leu Phe Arg Thr Ser Cys Asn Thr Ser Arg Pro	
100 105 110	
GGC CGC TTA AGC AAC ATA ACT TGC TAC GAA ACA ATG ATG GAT GCC ACT	382
Gly Arg Leu Ser Asn Ile Thr Cys Tyr Glu Thr Met Met Asp Ala Thr	
115 120 125	
ACG AAG CTT CAA CAA GTC ATC CTC ATC GCC GTG GGA GTC TTT CTG TCG	430
Thr Lys Leu Gln Gln Val Ile Leu Ile Ala Val Gly Val Phe Leu Ser	
130 135 140	
CTG GCG GCG CTG GCG GGG GGC TGT TTC TTC CTG GTG CTG AGA TAC AAA	478
Leu Ala Ala Leu Ala Gly Gly Cys Phe Phe Leu Val Leu Arg Tyr Lys	

145	150	155	
GGC CTG GTG AAA TAC TGG TTT CAC TCT CCG CCA AGC ATC CCA TCA CAA			526
Gly Leu Val Lys Tyr Trp Phe His Ser Pro Pro Ser Ile Pro Ser Gln			
160	165	170	175
ATC GAA GAG TAT CTG AAG GAC CCG AGC CAG CCT ATC CTA GAG GCC CTG			574
Ile Glu Glu Tyr Leu Lys Asp Pro Ser Gln Pro Ile Leu Glu Ala Leu			
180	185		190
GAC AAG GAC ACG TCA CCA ACA GAT GAT GCC TGG GAC TTG GTG TCT GTT			622
Asp Lys Asp Thr Ser Pro Thr Asp Asp Ala Trp Asp Leu Val Ser Val			
195	200		205
GTT GCA TTT CCA GCA AAG GAG CAA GAA GAT GTT CCC CAA AGC ACT TTG			670
Val Ala Phe Pro Ala Lys Glu Gln Glu Asp Val Pro Gln Ser Thr Leu			
210	215		220
ACC CAA AAC TCT GGT GCG GTC TGC TAGCCTGTGG GGTAAGGGCT CTGAGCCGAG			724
Thr Gln Asn Ser Gly Ala Val Cys			
225	230		
GAAGCTGCTG ATGTCCATGT CAGCACTTTA TGGAATCCGG TCCTCCATTT TCCTGTCCCC			784
AAAAGGCCCG TCAGTGCCTG TGAAGATGTA ACGGGTCTCA TGGGGGCGAC AAGCTTATTG			844
ATTTTTTCT TCAAATAAG AGTTTTCTAA TCATACGCGT TTTTAGAATA ATTCTACAGA			904
TATGTCCCCG AAAGATTAAAG ATTTCTCTTA AACACTAAAA AGACATGTAA TTATTTGTTA			964
GCAAATGGGC GTCTGGCACG CCTCTGACAC TTTTTCGTCA GCAGCCAGGA CACGAGGTCC			1024
CCTCCTTGAT GAAGCCCCCTC GGGCAGACCA TGTCACCTGT CCCAGCCTGC CCCAAGAAGG			1084
GACATTAAGT GGCCCTTCTT CATATCCAAA CACCTGGCTT GAAATGTGAT TAGCCCTGTA			1144
AATAGTTTCA CAGAGATTAA GCCTTTTTTTT CCCCCAAGTT AGGAATAAAA GACTATAATT			1204
AACTTTTAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA			1244

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 231 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Arg	Val	Asp	Pro	Arg	Val	Arg	Leu	Val	Ser	Pro	Trp	Leu	Thr	Val	Pro
1				5					10					15	

Trp	Phe	Leu	Ser	Cys	Trp	Asn	Val	Thr	Ile	Gly	Pro	Pro	Glu	Ser	Ile	
			20									25		30		
Trp	Val	Thr	Pro	Gly	Glu	Ala	Ser	Leu	Ile	Ile	Arg	Phe	Ser	Ser	Pro	
			35									40		45		
Phe	Asp	Val	Pro	Pro	Asn	Leu	Gly	Tyr	Phe	Gln	Tyr	Tyr	Val	His	Tyr	
			50									55		60		
Trp	Glu	Lys	Ala	Gly	Ile	Gln	Lys	Val	Lys	Gly	Pro	Phe	Lys	Ser	Asn	
			65									70		75		
Ser	Ile	Val	Leu	Asp	Gly	Leu	Arg	Pro	Leu	Arg	Glu	Tyr	Cys	Leu	Gln	
			85									90		95		
Val	Lys	Ala	His	Leu	Phe	Arg	Thr	Ser	Cys	Asn	Thr	Ser	Arg	Pro	Gly	
			100									105		110		
Arg	Leu	Ser	Asn	Ile	Thr	Cys	Tyr	Glu	Thr	Met	Met	Asp	Ala	Thr	Thr	
			115									120		125		
Lys	Leu	Gln	Gln	Val	Ile	Leu	Ile	Ala	Val	Gly	Val	Phe	Leu	Ser	Leu	
			130									135		140		
Ala	Ala	Leu	Ala	Gly	Gly	Cys	Phe	Phe	Leu	Val	Leu	Arg	Tyr	Lys	Gly	
			145									150		155		
Leu	Val	Lys	Tyr	Trp	Phe	His	Ser	Pro	Pro	Ser	Ile	Pro	Ser	Gln	Ile	
			165									170		175		
Glu	Glu	Tyr	Leu	Lys	Asp	Pro	Ser	Gln	Pro	Ile	Leu	Glu	Ala	Leu	Asp	
			180									185		190		
Lys	Asp	Thr	Ser	Pro	Thr	Asp	Asp	Ala	Trp	Asp	Leu	Val	Ser	Val	Val	
			195									200		205		
Ala	Phe	Pro	Ala	Lys	Glu	Gln	Glu	Asp	Val	Pro	Gln	Ser	Thr	Leu	Thr	
			210									215		220		
Gln	Asn	Ser	Gly	Ala	Val	Cys										
			225									230				

(2) INFORMATION FOR SEQ ID NO:5:

(ii) MOLECULE TYPE: peptide

Tyr Arg Gly Leu Ile Lys Tyr Trp Phe His Thr Pro Pro Ser Ile Pro
 275 280 285

Leu Gln Ile Glu Glu Tyr Leu Lys Asp Pro Thr Gln Pro Ile Leu Glu
 290 295 300

Ala Leu Asp Lys Asp Ser Ser Pro Lys Asp Asp Val Trp Asp Ser Val
 305 310 315 320

Ser Ile Ile Ser Phe Pro Glu Lys Glu Gln Glu Asp Val Leu Gln Thr
 325 330 335

Leu

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 325 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ala Trp Ser Leu Gly Ser Trp Leu Gly Gly Cys Leu Leu Val Ser
 1 5 10 15

Ala Leu Gly Met Val Pro Pro Pro Glu Asn Val Arg Met Asn Ser Val
 20 25 30

Asn Phe Lys Asn Ile Leu Gln Trp Glu Ser Pro Ala Phe Ala Lys Gly
 35 40 45

Asn Leu Thr Phe Thr Ala Gln Tyr Leu Ser Tyr Arg Ile Phe Gln Asp
 50 55 60

Lys Cys Met Asn Thr Thr Leu Thr Glu Cys Asp Phe Ser Ser Leu Ser
 65 70 75 80

Lys Tyr Gly Asp His Thr Leu Arg Val Arg Ala Glu Phe Ala Asp Glu
 85 90 95

His Ser Asp Trp Val Asn Ile Thr Phe Cys Pro Val Asp Asp Thr Ile
 100 105 110

Ile Gly Pro Pro Gly Met Gln Val Glu Val Leu Ala Asp Ser Leu His
 115 120 125

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Met Arg Phe Leu Ala Pro Lys Ile Glu Asn Glu Tyr Glu Thr Trp Thr
130 135 140

Met Lys Asn Val Tyr Asn Ser Trp Thr Tyr Asn Val Gln Tyr Trp Lys
145 150 155 160

Asn Gly Thr Asp Glu Lys Phe Gln Ile Thr Pro Gln Tyr Asp Phe Glu
165 170 175

Val Leu Arg Asn Leu Glu Pro Trp Thr Thr Tyr Cys Val Gln Val Arg
180 185 190

Gly Phe Leu Pro Asp Arg Asn Lys Ala Gly Glu Trp Ser Glu Pro Val
195 200 205

Cys Glu Gln Thr Thr His Asp Glu Thr Val Pro Ser Trp Met Val Ala
210 215 220

Val Ile Leu Met Ala Ser Val Phe Met Val Cys Leu Ala Leu Leu Gly
225 230 235 240

Cys Phe Ser Leu Leu Trp Cys Val Tyr Lys Lys Thr Lys Tyr Ala Phe
245 250 255

Ser Pro Arg Asn Ser Leu Pro Gln His Leu Lys Glu Phe Leu Gly His
260 265 270

Pro His His Asn Thr Leu Leu Phe Phe Ser Phe Pro Leu Ser Asp Glu
275 280 285

Asn Asp Val Phe Asp Lys Leu Ser Val Ile Ala Glu Asp Ser Glu Ser
290 295 300

Gly Lys Gln Asn Pro Gly Asp Ser Cys Ser Leu Gly Thr Pro Pro Gly
305 310 315 320

Gln Gly Pro Gln Ser
325

668660-0488866

[illegible]

As a below-named inventor, I hereby declare that:

I believe I am the original, first sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

the specification of which

1 was filed on March 8, 1999 as Application Serial No. to be assigned,

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

(Number)	(Country)	(Day/Month/Year Filed)	Yes or No
----------	-----------	------------------------	-----------

<u>60/077,329</u> (Application Number)	<u>March 9, 1998</u> (Filing Date)
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(Application Serial No.)	(Filing Date)	(Status – patented, pending, abandoned)
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Page 1 of 3

Power of Attorney: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in Patent and Trademark Office connected therewith. (List name and registration number.)

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I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of First Inventor	Signature of Second Inventor	Signature of Third Inventor
Christi L. Parham	Kevin W. Moore	Nicholas J. Murgolo
Date:	Date:	Date:

Signature of Fourth Inventor
J. Fernando Bazan
Date: